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CONTENTS

PAPER PARTITION CHROMATOGRAPHIC STUDIES OF URINARY AMINO ACIDS IN HUMAN LIVER DISEASE

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The liver is known to be the most important site of amino acid metabolism (Mann 1927). Disturbances of amino acid metabolism may therefore be anticipated in liver diseases. The classical observation of the presence of leucine and tyrosine crystals in the urine of human subjects with acute yellow atrophy lends support to this possibility. However chemical estimation of serum alpha amino nitrogen in cases of cirrhosis and hepatitis have shown the levels to be normal in such subjects (Lyttle *et al* 1943, de Vries and Alexander 1948). Moreover due to the enormous reserve power of the liver significant rises in blood amino acid levels are not seen even after as much as 90% of the liver has been removed, when these are measured by routine chemical methods. Using microbiological methods increased urinary excretion of only 2 amino acids (methionine and tryptophane) could be demonstrated (Dunn *et al* 1950, Gabuzda *et al* 1952). These observations make routine chemical and microbiological methods of amino acid analysis relatively useless in the study of amino acid changes in mild or moderately severe liver disease.

The recent development of paper partition chromatography as an analytical tool has led to its utilisation in the study of amino acid abnormalities in liver disorders. In blood increased levels of individual amino acids such as methionine (Kirmer *et al* 1950), glycine (de Vries and Alexander loc. cit.) and glutamine and tyrosine (Walshe 1951, 1953) have been reported to occur in experimental and human liver disease. In non fatal *hepatitis or cirrhosis considerably more amino acids than normal are excreted* in urine particularly tyrosine, cystine and glutamine (Dent and Walshe 1951). *Wahi et al (1955) have demonstrated* marked increases in liver free amino acids following acute hepatic injury by carbon tetrachloride. *Dutta (1954) studied by paper chromatographic technique urinary* amino acid excretion in a variety of diseases and found that in liver disease *the number and concentration of amino acids was considerably* more than normal limits.

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Dent and Walshe (1951) and Walshe (1951 1953) have claimed that marked qualitative changes in amino acid excretion occur only in acute yellow atrophy of the liver and in hepatic coma while in hepatitis and cirrhosis only minor changes occurred There have been no reports of quantitative analysis of amino acids in these diseases. The present study was undertaken to find out the quantitative changes in amino acid excretion both in mild and severe cirrhosis and hepatitis and also to establish the amino acid excretion pattern if any characteristic of these 2 diseases

Material and Methods

A total of 25 cases of portal cirrhosis and 20 cases of infectious hepatitis were studied The cases were in the wards of S N Hospital Agra. Twenty four-hour urine specimens of these patients were separately collected under a layer of toluene and stored in the refrigerator after measuring their total volume.

For qualitative analysis amino acids in the urine specimens were separated by the double dimensional paper partition chromatographic technique described earlier (Wahi and Nigam 1954) using phenol as the first solvent and butanol acetic acid water as the second solvent. An aliquot of 0 025 cc of urine was applied on Whatman filter paper No 1 for the analysis. The amino acids were detected by spraying paper with 0.25% ninhydrine in butyl alcohol

For quantitative analysis the circular chromatographic technique described earlier (Wahi *et al* 1954) was employed with butanol-acetic-acid water as the solvent. After development and spraying of the chromatogram the colours of amino acid spots with ninhydrine were measured in Klett Summerson photo-electric colorimeter An aliquot of 0 05 cc of urine was used for analysis

Observations and Results

Amino acid pattern

The urinary amino acid-excretion pattern for normal human subjects is shown in Fig I It will be seen that the normal chromatographic pattern consists of the four amino acids-glycine serine alanine and glutamine Of these the glycine spot was the strongest. This pattern is similar to the one reported for healthy human subjects in western countries (Dent and Walshe 1954)

Fig II shows the typical chromatographic pattern for cases of cirrhosis and infectious hepatitis. Compared to the normal pattern several more amino acids are also present on the chromatogram. The amino acids visible are cystine, glycine serine alanine glutamine valine leucine glutamic acid and methionine Also the intensity of the glycine serine alanine and

glutamine spots were moderately to markedly increased In a few cases aspartic acid spot was also visible on the chromatogram

Urinary Excretion of Amino acids (Quantitative)

Table I gives the data for urinary excretion of the different amino acid in normal human subjects and in patients with portal cirrhosis It will be observed that compared to normal excretion figures for healthy subjects there is generally an elevated amino acid excretion in the cirrhosis patients Glycine serine glutamine and cystine were consistently increased but this was not so with glutamic acid and valine

Table II shows the urinary excretion of various amino acids in cases of infectious hepatitis compared to normal human individuals As in the cases of portal cirrhosis glycine serine, alanine and glutamine showed the most consistent increases. Methionine glutamic acid and valine were only occasionally increased. Cysteic acid excretion was almost invariably elevated.

Discussion

The results obtained in the present study show that increased excretion of certain amino acids do occur in practically all cases of portal cirrhosis and infectious hepatitis irrespective of the severity of the disease To this extent, therefore paper chromatographic studies of urinary amino acids serves as a test of liver function and could well be used in conjunction with other liver function tests However due to the prolonged time involved in completing the chromatographic process (72 hours) the practical importance of this test is rather diminished in this respect

The finding that methionine and cystine are often excreted in increased amounts in cirrhosis and hepatitis cases is of considerable significance Methionine is known to be the precursor of the important lipotropic (and anti-cirrhotic) factor choline so that increased urinary excretion of methionine suggests its non utilisation for choline synthesis. Cystine is one of the factors protective against liver necrosis and its increased urinary excretion is indicative of a defect in its utilisation by the body

The mechanism causing the excessive excretion of amino acids in the urine cannot be definitely stated However since the liver is the principal site of degradation of amino acids (deamination) it is highly probable that defective deamination leads to rise in plasma levels of amino acids with consequent higher urinary excretion Dent and Walshe (1954) suggest that the amino-aciduria in liver disease is the overflow type unlike the renal type of amino-aciduria that occurs in Wilson's disease DeToni-Fanconi syndrome and galactosemia That defective deamination occurs in liver disorders is shown by the fact that low blood urea levels occur concurrently with high blood amino acid levels (Nonnenbruch, 1939 Switzer et al 1952) in severe hepatic insufficiency though the blood urea level are often also

elevated owing to the renal injury associated with hepatic disorders (Wilensky 1947)

In addition to defective deamination of amino acids it is also likely that the necrosis of liver cells results in release into blood stream of amino acids which, in conjunction with the impaired deaminating power of liver lead to further rise in plasma amino acids and consequent amino-aciduria. This mechanism will be particularly important in infective hepatitis.

Kinsell *et al* (1950) have demonstrated that *cirrhotic patients had im*paired ability to incorporate methionine labelled with S^{35} into the plasma proteins These together with the high methionine and cystine levels in blood and in urine (Table I) seen in such subjects suggest that the amino aciduria is also due to diminished protein synthesis.

Wahl *et al* (1956 1957 1960 1960) and Wahi and Ramachandran (1958) have shown that impaired secretion of adrenal cortical hormones occurs in experimental and human liver disease Since these hormones are known to promote deamination of amino acids, it is thus also probable that diminished adrenal cortical function is yet another factor causing the amino-aciduria of liver disease. Recently obtained data (Wahl *et al* 1959) tend to support this hypothesis

The finding in the present study that amino-aciduria is almost a constant finding in acute and chronic liver disease is of considerable clinical and therapeutic significance. In the first place this shows clearly that there *is no amino acid deficiency in these subjects.* The implication of this finding would be that amino acid therapy is almost valueless and even harmful in these patients for they develop hepatic coma with almost always fatal results. This is because these subjects have apparently lost their capacity to effectively utilise or destroy these amino acids. Particularly their ability *to metabolise* the key amino acid methionine is impaired (Kirsner *et al*, 1950) Hence protein hydrolysates or amino acid mixtures should not be administered to these patients. Davidson and Gabuzda (1950) have in fact, concluded from a critical review of the extensive literature on this aspect that there is no valid evidence that addition of choline or methionine to a nutritious diet induces a more rapid more complete or more certain recovery than diet alone, in the cirrhotic patient.

SUMMARY

1 Urinary amino acid excretion was studied in 25 cases of cirrhosis and 20 cases *of infectious hepatitis* by the double dimensional and circular paper partition chromatographic techniques.

2 Increased amino acid excretion was observed in almost all the cases of portal cirrhosis and infectious hepatitis. The urinary amino acid pattern in these cases was markedly different from those seen in normal human subjects.

3 The amino acids that most often showed increases on the chromatogram were cystine glycine, serine, alanine glutamine, methionine, valine and leucine

4 It is suggested that (i) excessive release of amino acids from liver cell necrosis (ii) defective deamination of the liberated amino acids by the damaged liver and (iii) impaired protein synthesis, all lead to accumulation of amino acids in plasma with consequent amino-aciduria.

Table 1

Urinary Amino Acid Excretion in Subjects with Portal Cirrhosis

			AMINO ACIDS				
Case No	Cystine	Glycine Serine-Aspartic Acid	Alanine	Glutamine	Methionine	valine	Leucine
			(MG Excreted per 24 Hours)†				
1	123	2112	212	243	21	41	38
2.	98	1484	188	273	32	52	32
3.	148	1711	314	315	28	39	48
4	212	1884	262	278	26	32	42
5	78	882	289	188	43	26	31
6	152	1564	268	414	61	42	46
7	161	1812	302	317	39	33	44
8.	158	2118	363	287	29	38	49
9	147	1600	288	302	32	42	50
10	189	1712	411	286	42	38	46
11	315	1861	388	292	31	40	37
12.	68	812	165	318	17	30	41
13.	212	1555	812	334	32	38	39
14	141	1564	406	412	27	48	43
15	89	1124	294	286	32	42	36
16	166	2122	246	324	26	31	37
17	214	2612	312	267	43	39	32
18.	182	1443	416	387	41	31	61
19	206	988	289	292	29	39	52
20	108	1221	333	236	40	48	36
21	121	1892	387	203	46	47	61
22	302	2122	404	612	52	51	63
23	188	1666	312	413	32	36	47
24	171	1584	288	382	34	40	39
25	264	1801	303	414	38	48	44

Expressed in terms of glycine

† NORMAL EXCRETION VALUES (AVERAGES)—Cystine 85 mg; Glycine-Serine Aspartic acid 750 mg Alanine 167 mg Glutamine 145 mg; Methionine 12.0 mg; Valine 20 mg Leucine 24 mg

elevated owing to the renal injury associated with hepatic disorders (Wilensky 1947)

In addition to defective deamination of amino acids it is also likely that the necrosis of liver cells results in release into blood stream of amino acids which in conjunction with the impaired deaminating power of liver lead to further rise in plasma amino acids and consequent amino-aciduria This mechanism will be particularly important in infective hepatitis.

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9. Kinsner, J. B., Sheffner, A. L., Palmer, W. L., & Bergeim, O. : *J. Lab. Clin. Med.*, 36 : 735, 1950.

10. Lytle, J. D., Goettsch, E., Greeley, D. M., Grim, W. M., & Dunbar, P. : *J. Clin. Invest.*, 22 : 169, 1943.

11. Mann, F. C. : *Medicine Baltimore*, 6 : 419, 1927.

12. Nonnenbruch, W. : *Clin. Wchschr.*, 18 : 917, 1939.

13. Russell, J. A., & Wilhelmi, A. E. : *J. Biol. Chem.*, 140 : 747, 1941.

14. Sulzer, J. L., Steigmann, F., de la Huerga, J., & Schaffner, F. : *Am. J. Dig. Dis.*, 19 : 41, 195...

15. Wahi, P. N., & Nigam, R. G. S. : *Ind. J. Med. Res.*, 42 : 761, 1954.

16. Wahi, P. N., Nigam, R. G. S., & Bharadwaj, T. P. : *Ind. J. Med. Sc.*, 8 : 427, 1954.

17. Wahi, P. N., Tandon, H. D., & Bharadwaj, T. P. : *Acta Path. et Microbiol. Scand.*, 37 : 315, 1955.

18. Wahi, P. N., Tandon, H. D., & Bharadwaj, T. P. : *A.M.A. Arch. Path.*, 62 : 200, 1956.

19. Wahi, P. N., Ramachandran, S., & Narani, H. : *J. Ind. Med. Assoc.*, 29 : 133, 1957.

20. Wahi, P. N., & Ramachandran, S. : *A.M.A. Arch. Path.*, 66 : 482, 1958.

21. Wahi, P. N., Ramachandran, S., & Kehar, U. : *Ind. J. Med. Sc.*, 14 : 302, 1960.

22. Wahi, P. N., Ramachandran, S., & Kehar, U. : Unpublished data (1959).

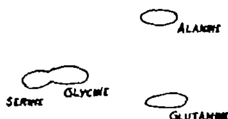
23. Wahi, P. N., Ramachandran, S., and Kehar, U. : *J. Path. Bact.*, 79 : 159, 1960.

24. Walshe, J. M. : *Quart. J. Med.*, n. s., 20 : 421, 1951.

25. Walshe, J. M. : *Ibid.* n. s., 22 : 483, 1953.

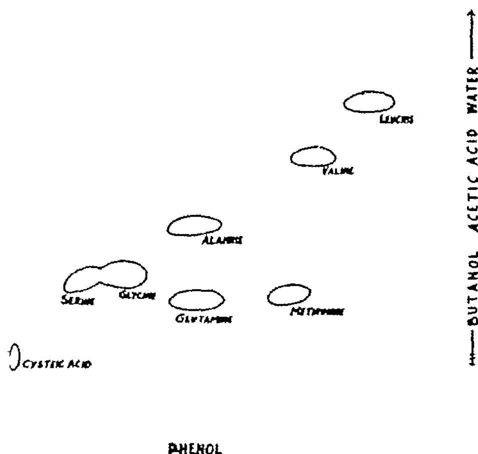
26. Wilensky, A. O. : *Am. J. Dig. Dis.*, 14 : 74, 1947.

FIGURE I :— URINARY AMINO ACID CHROMATOGRAPHIC PATTERN IN NORMAL HUMAN SUBJECTS



PHENOL

FIGURE II - URINARY AMINO ACID CHROMATOGRAPHIC PATTERN IN SUBJECTS WITH PORTAL CIRRHOSIS AND INFECTIOUS HEPATITIS



A GENERALIZED STIELTJES TRANSFORM

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CHAPTER ONE

Meijer C. S., Boas, R. P., Varma R. S. and Saksena K. M. have given generalizations of the Laplace integral

$$(1.1) \qquad f(s) = \int_0^{\infty} e^{-st} \phi(t) dt$$

Meijer has given two generalizations, given below

$$(1.2) \quad f(s) = (2/\pi)^{\frac{1}{2}} \int_0^{\infty} K_{\nu}(st)\,(st)^{\frac{1}{2}}\,\phi(t)\,dt$$

and

$$(1.3) \quad f(s) = \int_0^{\infty} e^{-\frac{1}{2}st}\, W_{k+\frac{1}{2},m}(st)\,(st)^{-k-\frac{1}{2}}\,\phi(t)\,dt$$

Varma has introduced the following two generalizations

$$(1.4) \quad f(s) = \int_0^{\infty} (2st)^{-\frac{1}{2}}\, W_{k,m}(2st)\,\phi(t)\,dt$$

and

$$(1.5) \quad f(s) = \int_0^{\infty} e^{-\frac{1}{2}st}\,(st)^{m-\frac{1}{2}}\, W_{k,m}(st)\,\phi(t)\,dt$$

On iterating the transform (1.1) we obtain the Stieltjes transform

$$(1.6) \qquad f(s) = \int_0^{\infty} (s+t)^{-1}\,\phi(t)\,dt$$

Varma gave two generalizations of (1.6) in the forms

$$(1.7) \quad f(s) = \Gamma(2m+1)\Gamma^{-1}(m-k+3/2)\,s^{-1} \int_0^{\infty} F(2m+1,1,m-k+3/2,-t/s)\,\phi(t)\,dt$$

and

$$(1.8) \quad f(s) = \Gamma(2m+1)\Gamma^{-1}(m-k+3/2) \int_0^{\infty} t^{-1}\, F(2m+1,1,m-k+3/2,-s/t)\,\phi(t)\,dt$$

Both (1.7) and (1.8) reduce to (1.6) when $k+m=\frac{1}{2}$. The transform (1.7) also reduces to another generalization of Stieltjes transform

* This is an abstract of the thesis submitted for the Ph. D. degree of the Agra University in the year 1958-59

(1 9) $\theta(s)=\int_0^{\infty}(s+t)^{-\rho}\,\phi(t)\,dt$

when $k-m=\frac{1}{2}$ $2m+1=\rho$ By suitable change of variables (1 7) may be reduced to (1 8) and vice-versa

Saksena K. M. and Snehlata have also given a number of generalizations of the Stieltjes transform by iterating different generalization of Laplace transform

We have studied the transform (1 7) in detail and have also found out some properties of the other generalization (1 8)

CHAPTER TWO

In this chapter convergence theorems asymptotic and order properties of the Stieltjes integrals

(2 1) $f(s)=\Gamma(2m+1)\,\Gamma^{-1}(m-k+3/2)\,s^{-1}\int_0^{\infty}F(2m+1\ 1\ m-k+3/2\ -t/s)\,d\alpha(t)$

and

(2.2) $f(s)=\Gamma(2m+1)\,\Gamma^{-1}(m-k+3/2)\,s^{-1}\int_{0+}^{\infty}F(2m+1\ 1\ m-k+3/2\ -t/s)\,d\alpha(t)$

are discussed. Some theorems are stated below (without proof) In all these theorems we assume that Re $(2m+1)>0$ and $m-k+3/2\neq 0\ -1$

Theorem 2 1 If the integral (2 1) converges for a point $s=s_0$, not on the negative real axis, $\sigma\leq 0$ $T=0$ then it converges for every such point.

Theorem 2·2 If the integral (2 1) converges, it converges uniformly in any closed bounded region not containing a point of the negative real axis, $\sigma\leq 0$, $T=0$

Theorem 2.3 If either of the integrals (2.1) and (2 2) converges, then

(2.3a) $\alpha(t)=o(t)$ $(t\to\infty)$

if (i) $2m$ is a positive integer

or (ii) $2m\neq 0$ or a positive integer $k\pm m\neq\frac{1}{2}$ Re $m>0$

or (iii) $k+m=\frac{1}{2}$

(2.3b) $\alpha(t)=o(t^{2m+1})$ $(t\to\infty)$

if (i) $k-m=\frac{1}{2}$

or (ii) $2m\neq 0$ or a positive integer Re $m<0$ and

(2.3c) $\alpha(t)=o(t/\log t)$ $(t\to\infty)$

if $2m=0$ and $-k+\frac{1}{2}\neq 0$

Theorem 2 4 If either of the integrals (2 1) and (2 2) converges, then $\alpha(0+)$ exists.

Theorem 2.5. If for some positive δ

(2.4a) $\alpha(t) = O(t^{1-\delta})$ $(t \to \infty)$

if (i) $2m$ is positive integer

or (ii) $2m \neq 0$ or positive integer $k \pm m \neq \frac{1}{2}$ Re $m \geq 0$

or (iii) $k + m = \frac{1}{2}$

(2.4b) $\alpha(t) = O(t^{2m+1-\delta})$ $(t \to \infty)$

if (i) $k - m = \frac{1}{2}$

or (ii) $2m \neq 0$ or positive integer $k \pm m \neq \frac{1}{2}$ Re $m < 0$

(2.4c) $\alpha(t) = O(t^{1-\delta}/\log t)$ $(t \to \infty)$

if $2m = 0$ $-k + \frac{1}{2} \neq 0$

then (2.1) converges. If in addition $\alpha(0+)$ exists then (2.2) converges.

Theorem 2.6a. If $f(s)$ is defined by convergent integral (2.1) with $\alpha(0) = 0$ and

(a) $2m$ is zero or positive integer

(b) $2m \neq 0$ or a positive integer $k \pm m \neq \frac{1}{2}$ Re $m \geq 0$

(c) $k + m = \frac{1}{2}$

then

(2.5) $f(s) \sim \alpha(0+)\, s^{-1}\, \Gamma(2m+1)\, \Gamma^{-1}(m-k+3/2)$ $(s \to 0+)$

(2.6) $f(s) = o(1)$ $(s \to \infty)$

Theorem 2.6b. If $f(s)$ is defined by convergent integral (2.1) with $\alpha(0) = 0$ and

(a) $k - m = \frac{1}{2}$

or (b) $2m \neq 0$ or a positive integer $k \pm m \neq \frac{1}{2}$ Re $m > 0$

(c) $2m$ is positive integer

then

(2.7) $f^{(n)}(s) \sim (-1)^n\, n!\, \Gamma(2m+1)\, \Gamma^{-1}(m-k+3/2)\, s^{-n-1}$ $(s \to 0$ $n = 1, 2, \ldots)$

(2.8) $f^{(n)}(s) = o(s^{-n})$ $(s \to \infty$ $n = 1, 2, \ldots)$

Theorem 2.7a. If $f(s)$ is defined by convergent integral (2.1) with $\alpha(0) = 0$, and

(a) $k - m = \frac{1}{2}$

or (b) $2m \neq 0$ or a positive integer $k \pm m \neq \frac{1}{2}$ Re $m < 0$ then

(2.9) $f(s) \sim \Gamma(2m+1)\, \Gamma^{-1}(m-k+3/2)\, \alpha(0+)\, s^{-1}$ $(s \to 0+)$

(2.10) $s^{-2m} f(s) = o(1)$ $(s \to \infty)$

Theorem 2.7b. If $f(s)$ is defined by (2.1) with $\alpha(0) = 0$, and

(a) $k - m = \frac{1}{2}$,

or (b) $2m \neq 0$ or positive integer $k \pm m \neq \frac{1}{2}$ Re $m < 0$ then

(2 11) $(d/ds)^n [s^{-2m} f(s)] \sim (-1)^n \Gamma(2m+n+1) \Gamma^{-1}(m-k+3/2)$
$s^{-n-1-2m}$ $(s \to 0+ \;\; n=1, 2, \ldots)$

(2 12) $(d/ds)^n [s^{-2m} f(s)] = o(s^{-n})$ $(s \to \infty \;\; n=1, 2, \ldots)$

We have also found out similar theorems giving the asymptotic properties of the transform (2.2)

Chapter Three

In this chapter the following Abelian theorem for the transform (2.1) is established We assume s as real and positive.

Theorem. If $f(s)$ is defined by convergent integral (2 1) for $s>0$ and if

(i) Re $(2m+1)>0$ $m-k+3/2 \neq 0, -1, -2,$

(ii) Re $(m-k+\frac{1}{2}+\gamma)>0$ Re $(\frac{1}{2}-m-k+\gamma)>0$, Re $(m+k+3/2-\gamma)>0$ then for any constant A

(3 1) $$\overline{\lim_{\substack{s\to 0+\\ s\to\infty}}} \; | \Gamma(m-k+3/2)\, \Gamma^{-1}(2m+1)\, s^{-m-k+\frac{1}{2}} f(s) - A | \leq$$

$$\leq \overline{\lim_{\substack{t\to 0+\\ t\to\infty}}} \left| \alpha(t)\, c^{-1}\, t^{-m-k-\frac{1}{2}+\gamma} - A \right|$$

where

$$c = \frac{\Gamma(2m+1)\; \Gamma(-2k+1+\gamma)}{\Gamma(m-k+3/2)\; \Gamma(-m-k+\frac{1}{2}+\gamma)\; \Gamma(\frac{1}{2}-m-k+\gamma)\; \Gamma(m+k+3/2-\gamma)}$$

Chapter Four

In this chapter we give two complex inversion theorems for the transform (1 7)

Theorem 4 1 If $f(s)$ is defined by convergent integral (1 7) then

(4 1) $$\tfrac{1}{2}[\phi(t+)+\phi(t-)] = (2\pi i)^{-1} \lim_{T\to\infty} \int_{c-iT}^{c+iT} \frac{\Gamma(m-k+\frac{1}{2}+l)}{\Gamma(2m+l)\Gamma(l)\Gamma(1-l)}\; t^{-l}\, \phi(l)\, dl$$

where

$$\phi(l) = \int_0^\infty s^{l-1} f(s)\, ds$$

provided that

(i) $x^{c-1} \phi(x) \in L(0, \infty)$

(ii) $x^{l-1} f(x) \subset L(0, \infty)$ $(l = c + iT, -\infty < T < \infty)$

(iii) $\phi(x)$ is of bounded variation in the neighbourhood of the point $x = t$ $(t > 0)$

(iv) $\phi(t) = 0(t^{\rho})$ $\quad$ Re $(\rho) > 0$ $\quad (t \to 0)$

$= 0(t^{-\gamma})$ Re $\gamma > 0$ $\quad (t \to \infty)$

(v) $1 > \text{Re}\, l > 0$ Re $(2m + l) > 0$ $m - k + 3/2 \neq 0, -1, -2$

Theorem 4.2. If $f(s)$ is defined by convergent integral (1) then

$$(4\ 2)\quad \phi(t) = (\pi i)\, t^{m-1} \int_{c-i\infty}^{c+i\infty} G(y)\, I_{2m}(y t^{-\frac{1}{2}})\, y^{\frac{1}{2}}\, dy$$

where

$$G(y) = \int^{\infty} A(s, y)\, f(s)\, ds$$

and

$$A(s, y) = 2^{m+k-2}\, y^{1-m-l}\, s^{\frac{1}{2}(k-m)-\frac{3}{4}}\, J_{m-k+\frac{1}{2}}(y s^{-\frac{1}{2}})$$

provided that

(i) $0 < \text{Re}\,(m - k + 3/2) > 2 \max [\text{Re}\,(2m+1), 1] - \frac{1}{2}$

(ii) Re $(2m + 1) > 0$,

(iii) $\int^{\infty} t^{-\delta} \phi(t)\, dt$ converges where $0 \leq \delta < \min [\text{Re}\,(2m+1), 1]$

(iv) the functions $\phi(u)$ and $G(y)$ are such as to permit the complex inversion of the transform

$$G(y) = \int_{0}^{\infty} K_{2m}(yu)\, (yu)^{\frac{1}{2}}\, u^{2m-\frac{1}{2}}\, \phi(u^{-2})\, du$$

The *theorems* are *illustrated* by examples. The particular cases give corresponding results for (1.6) and (1.9)

CHAPTER FIVE

In this chapter we give a real inversion theorem for the transform (1 7) by changing it into a K transform and applying a differential operator which serves to invert the changed transform. Also we establish the equivalence of our operator with that of Boas R. P

Definition 5 1 An operator $V_{x,n}^{r}$ [H ( )] is defined for any real positive x and any positive integer n by

(5.1) $$V^{\gamma}_{n,n}[H(s)] = \frac{(2n)^{\frac{1}{2}-n\gamma}}{(2n)!} s^{2n+2\gamma} (s^{-1} d/ds)^n s^{2n-2\gamma} (s^{-1} d/ds)^n s^{\gamma-\frac{1}{2}} H(s) \Big|_{s=2n\,x^{\frac{1}{2}}}$$

Theorem 5.1 If $f(s)$ is defined by (1.7) then

(5.2) $$V^{\gamma}_{n,n}[H(y)] \sim \phi(x) \qquad (n \to \infty)$$

where

$$H(y) = \int_0^{\infty} B(s, y)\ f(s)\ ds$$

and

$$B(s, y) = (\pi)^{-\frac{1}{2}}\ 2^{m+k-2}\ y^{1-m-k}\ s^{\frac{1}{2}(k-m)-3/4}\ J_{m-k+\frac{1}{2}}\ (ys^{-\frac{1}{2}})$$

provided that (i) $0 < \text{Re}\,(m-k+3/2) < 2 \max[\text{Re}\,(2m+1), 1] - \frac{1}{2}$

(ii) $\text{Re}\,(2m+1) > 0$ (iii) $\int^{A} t^{-\delta_1}\phi(t)\,dt$ and $\int_A^{\infty} t^{-\delta_2}\phi(t)\,dt$

converge where $0 \leq \delta_1, \delta_2 < \min[\text{Re}\,(2m+1), 1]$ (iv) $y^{2m-3/2}\,\phi(y^{-2}) \in L$ in $0 \leq y \leq R$ for every $R > 0$ (v) $H(y)$ is infinitely differentiable.

The theorem and its corollaries are illustrated by examples.

Chapter Six

In this chapter we find singular integrals which serve to invert the transforms (1.7) (1.8) (1.3) and (1.5). We first establish the following theorem.

Theorem 6.1 If (a) $a < a+\eta < b$ (b) $\beta(t) \in C^2\,(a \leq t \leq a+\eta)$ $\beta(a) = 0$, $\beta''(a) < 0$, $\beta(t)$ is non increasing $(a \leq t \leq b)$

(c) $A(t)$ is continuous in some right hand neighbourhood of $t=a$ and integrable in any finite interval and $A(a) \neq 0$

(d) $\phi(t) \in L\,(a \leq t \leq b)$, $\phi(a) \neq 0$

$$\alpha(t) = \int_a^t [\phi(u) - \phi(a)]\,du = o(t-a) \qquad (t \to a+)$$

then

(6.1) $$\int_a^b \phi(t)\,e^{n\beta(t)}\ A(t)\,dt \sim \phi(a) e^{n\beta(a)}\ A(a) \left(\frac{-\pi}{2n\,\beta''(a)}\right)^{\frac{1}{2}} (n \to \infty)$$

With the help of this theorem the following main theorems are established.

Theorem 6.2. If

(1) $\phi(t) \subset L\,(R^{-1} \leq t \leq R)$ for every $R > 1$

(2) $\int_1^{\infty} \phi(t)\, t^{c}\, dt$ converges for a fixed real constant c

(3) $\int_{+}^{1} \phi(t)\, t^{c'}\, dt$ converges for a fixed real constant c'

(4) $\int_s^t [\phi(u) - \phi(t)]\, du = o(t-s) \qquad (t \to s)$

then

(6·2) $$\frac{\Gamma(2n+m+k+\frac{1}{2})}{\Gamma(n+1)\,\Gamma(n+m+k-3/2)} \int_{+}^{\infty} \frac{s^{n+m+k-3/2}\, t^{n}}{(s+t)^{2n+m+k+\frac{1}{2}}}\, \phi(t)\, dt \sim \phi(s) \qquad (n \to \infty)$$

Theorem 6 3 If

(1) $\phi(t) \subset L\,(R^{-1} \leq t \leq R)$ for every $R > 1$

(2) $\int^{\infty} \phi(t)\; {}^{-ct}\, dt$ converges for a fixed positive c

(3) $\int_{+}^{1} \phi(t)\, t^{r}\, dt$ converges for a fixed r

(4) $\int_s^t [\phi(u) - \phi(s)]\, du = o(|s-t|) \qquad (t \to s)$

then

(6 3) $$\Gamma^{-1}(m+k+\tfrac{1}{2}+n)\,(n/\;)^{m+k+\frac{1}{2}+n} \int^{\infty} {}^{-nt/}\; t^{m+k+\frac{1}{2}+n}\, \phi(t)\, dt \sim \phi(s) \qquad (n \to \infty)$$

CHAPTER SEVEN

In this chapter inversion theorems for the transforms (1 7) (1.8) (1 3) and (1.5) are g ven The following integro-differential operator is defined for the transform (1 7)

Definition 7 1 An operator $L_{n,s}[f(s)]$ is defined for any real positi e by the equations

$$L_{n,s}[f(s)] = \frac{\Gamma(2n+m-k+\frac{1}{2})\,\Gamma(2n+m+k+\frac{1}{2})}{\Gamma(2n+2m)\,\Gamma(2n)\,\Gamma(n+1)\,\Gamma(n+m+k-3/2)}\,(-1)^{n-}$$

$$D^{n}\, s^{2n-1}\, D^{n-1}\, s^{2m+n-1}\, D^{n-1}\, s^{-k-m+\frac{1}{2}}\, D^{1-n}\, s^{-m+k-n+\frac{1}{2}} f(s)$$

$(n=2, 3, \ldots)$

$= f(s) \qquad (n=0)$

$= D[s f(s)]$ where $D \equiv (d/ds)$ $\quad D^{-1} s^{a} = \int_0^s s^a \, ds$ if $\mathrm{Re}(a+1)>0$

$$D^{-1} s^{a} = -\int_s^{\infty} s^a \, ds \text{ if } \mathrm{Re}\,(a+1)<0$$

We have the following inversion theorem for (1 7)

Theorem 7 1 If $\phi(t) \subset L$ in $0 \leq t \leq R$ for every positive R and is such that the integral (1 7) converges, then

(7 1) $\qquad L_{n,s}[f(s)] \sim \phi(s) \qquad (n \to \infty)$

at all points of s of the Lebesgue set for the function $\phi(t)$

Similarly we define another inversion operator for the transform (1 8) which serves to invert it.

We also consider the general Stieltjes integral (2 1) and establish the following theorem.

Theorem 7.2. If $\alpha(t)$ is a normalized function of bounded variation in $0 \leq t \leq R$ for every positive R and if the integral (2.1) converges then

(7.2) $\qquad L_{n,x}[f(x)]\, dx = \alpha(t) - \alpha(0+)$

In this chapter we also define inversion operators for the transforms (1 3) and (1 5) and establish inversion theorems for them also

CHAPTER EIGHT

In this chapter we give representation theorems for the transforms (1 7) and (2 1) The two main theorems are given below

Theorem 8.1 If $f(s)$ has derivatives and integrals of all orders in $0<s<\infty$ which satisfy the conditions

$$f^{(n)}(s) = o\,(s^{-n-1}) \qquad (s \to 0+ \;\; n=0, 1, 2, \ldots)$$

$$= o\,(s^{-n}) \qquad (s \to \infty \;\; n=0, 1, 2, \ldots)$$

then provided that $\mathrm{Re}\,(2m+1)>0$ $\; m-k+3/2 \neq 0, -1, -2, \ldots$

$$\lim_{n\to\infty} \frac{\Gamma(2m+1)}{t\,\Gamma(m-k+3/2)} \int_{0+}^{\infty} F\,(2m+1, 1, m-k+3/2, -s/t)\, L_{n,s}[f(t)]\, ds = f(t) \quad (0<t<\infty)$$

Definition 8.1 A function $f(x)$ satisfies conditions D if it has derivatives and integrals of all orders in $0<x<\infty$ and if there exists a constant M such that

$L_{n,s}[f(x)]\, ds < M \qquad (n=1, 2, \ldots)$

Theorem 8.2. A necessary and sufficient condition that

$$f(x)=\Gamma(2m+1)\,\Gamma^{-1}(m-k+3/2)x^{-1}\int^{\infty} F\,(2m+1\ \ 1\ \ m-k+3/2\ \ -t/x)\ d\alpha\ (t)$$

with $\alpha\ (t)$ of bounded variation in $(0\ \ \infty)$ is that $f\ (x)$ should satisfy Conditions D

CHAPTER NINE

In this chapter we treat (1 7) (1.8) (1.3) and (1.5) as convolution transforms. We show that the integro-differential operators for these transforms (as given in Chapter Seven) can be deduced from the inversion functions

CHAPTER TEN

In this chapter we use the inversion theorem for (1 8) to obtain two inversion theorems for the generalized Laplace transform (1.5)

Theorem 10 1 If $\phi\ (t) \subset L\ (0\ \ \infty)$ and $f\ (s)$ is defined by the convergent integral (1 5) then

$$\frac{(-1)^{n+1}\ n^{-m-k+\frac{1}{2}}}{(n+1)!\ (n-1)!}\int^{\infty} e^{-st}\, t^{n}\, s^{n+m-k+\frac{1}{2}}\, D^{-(n+1)}\, s^{m+k-\frac{1}{2}}$$

$$D^{n+1} s^{n+1-2m} f^{(n+1)}\ (\ )\ \ ds$$

$$\sim \phi\ (t) \qquad (n \to \infty)$$

The other theorem is similar to the theorem given above.

CHAPTER ELEVEN

In this chapter we give some theorems connected with the transform (1 7) They enable us to evaluate some infinite integrals. A generalization of the third iterate of the Laplace transform is also given in the form

$$(11\ 1)\quad g\ (u)=u^{-1}\int^{\infty} v^{-1}\, E\ (2m+1\ \ 1\ \ 1\ \ m-k+3/2\ \ uv)\ h\ (v)\ \ dv$$

which, when $k+m=\frac{1}{2}$ reduces to

$$(11.2)\quad g\ (u)=\int_{0}^{\infty} e^{-uv}\,\Gamma\ (0\ \ uv)\ h\ (v)\ dv$$

which is discussed by Akutowicz E. J

The following are main theorems of this chapter

Theorem 11 1 If

$$(11.3)\quad g\ (u)=\Gamma(2m+1)\ \Gamma^{-1}(m-k+3/2)\ u^{-1}\int^{\infty} F\ (2m+1\ \ 1\ \ m-k+3/2\ -\ /u)$$

$$\phi\ (s)\ ds$$

and

$(n=2, 3 \ \ )$

$= f(s) \qquad (n=0)$

$= D[s f(s)]$ where $D \equiv (d/ds)$ $\quad D^{-1} s^a = \int_0^s s^a \, ds$ if $Re(a+1)>0$

$$D^{-1} s^a = -\int_s^{\infty} s^a \, ds \text{ if } Re(a+1)<0$$

We have the following inversion theorem for (1 7)

Theorem 7 1 If $\phi(t) \in L$ in $0 \leq t \leq R$ for every positive R and is such that the integral (1 7) converges then

(7 1) $\quad L_{n,s}[f(s)] \sim \phi(s) \qquad (n \to \infty)$

at all points of s of the Lebesgue set for the function $\phi(t)$

Similarly we define another inversion operator for the transform (1 8) which serves to invert it.

We also consider the general Stieltjes integral (2 1) and establish the following theorem.

Theorem 7.2. If $\alpha(t)$ is a normalized function of bounded variation in $0 \leq t \leq R$ for every positive R and if the integral (2.1) converges, then

(7.2) $\quad L_{n,x}[f(x)]\, dx = \alpha(t) - \alpha(0+)$

In this chapter we also define inversion operators for the transforms (1 3) and (1 5) and establish inversion theorems for them also

CHAPTER EIGHT

In this chapter we give representation theorems for the transforms (1 7) and (2 1) The two main theorems are given below

Theorem 8.1 If $f(s)$ has derivatives and integrals of all orders in $0<s<\infty$ which satisfy the conditions

$$f^{(n)}(s) = o(s^{-n-1}) \qquad (s \to 0+ \ \ n=0, 1, 2, \ \ )$$

$$= o(s^{-n}) \qquad (s \to \infty, n=0, 1, 2 \ \ )$$

then provided that $Re(2m+1)>0$ $\ m-k+3/2 \neq 0, -1, -2$

$$\lim_{n\to\infty} \frac{\Gamma(2m+1)}{t\,\Gamma(m-k+3/2)} \int_{0+}^{\infty} F(2m+1, 1, m-k+3/2, -s/t)\, L_{n,s}[f(t)]\, ds = f(t) \quad (0<t<\infty)$$

Definition 8.1 A function $f(x)$ satisfies conditions D if it has derivatives and integrals of all orders in $0<x<\infty$ and if there exists a constant M such that

$L_{n,s}[f(x)]\, ds < M \qquad (n=1, 2 \ \ )$

$$1 \cdot u \cdot b \quad \left| \int_0^{\infty} e^{s_0 t} \; (s_0 t)^{2m} \; \psi(\tfrac{1}{2}-k+m \;\; 2m+1 \;\; s_0 t) \; d\alpha\,(t) \right| = M < \infty$$
$0 \leq x < \infty$

then (1.5) converges for every s $(=\sigma+iT)$ for which $\sigma>\sigma_0$ $(s_0 = \sigma_0 + iT_0)$

provided that (i) $\frac{1}{2}-k+m \neq 0$ or a negative integer

or (ii) $\frac{1}{2}-k-m=0$, Re $m>0$

It is to be noted that

(12.1) $W_{k,m}(z) = e^{-\frac{1}{2}z} z^{m+\frac{1}{2}} \psi(\tfrac{1}{2}-k+m, 2m+1 \;\; z)$

We have found out a similar theorem for (1.3)

We also consider the necessary conditions imposed on $\alpha\,(t)$ by the convergence of these integrals. We here give two theorems for the integral (1.5)

Theorem 12.2. If the integral (1.5) converges for $s=s_0 = \gamma+i\delta$ with $\gamma > 0$, then

(12 2) $\alpha\,(t) = o(e^{\gamma t} t^{-k+m+\frac{1}{2}})$ $(t\to\infty)$

Theorem 12.3. If the integral (1 5) converges for $s=s_0 = \gamma+i\delta$ with $\gamma<0$, and if $\alpha(\infty)$ exists, then

(12.3) $\alpha(t)-\alpha\,(\infty) = o\,(e^{\gamma t} t^{-m-k+\frac{1}{2}})$ $(t\to\infty)$

We have similar theorems for the transform (1.3)

We have also established the following Abelian theorem for the transform (1 5)

Theorem 12.4 If $f\,(s)$ is defined by convergent integral (1.5) then for any constant A

$$\overline{\lim_{\substack{\to 0+ \\ s\to\infty}}} \left| s^{\gamma-k-m+\frac{1}{2}} f\,(s) - A \right|$$

$$\leq \overline{\lim_{\substack{t\to\infty \\ t\to 0+}}} \left| \frac{\alpha(t)\,\Gamma(\gamma-k-m+3/2)\;\Gamma(\gamma-k+\frac{1}{2}+m)}{\Gamma(\gamma-2k+1)\;\; t^{\gamma-k-m+\frac{1}{2}}} - A \right|$$

provided that

(1) Re $(\gamma-k+m+\frac{1}{2}) > 0$ Re$(\gamma-m-k+3/2)>0$

(2) $-k\pm(m-\frac{1}{2})\neq 0$ or a negative integer

(3) (i) $\alpha(t)=o(1)$ Re $m \geq 0$, $m\neq 0$, $(t\to 0)$

or (ii) $\alpha(t)=o\,(t^{-2m})$ Re $m<0$, $(t\to 0)$

or (iii) $\alpha(t)=o\,(1/\log t)$ $m=0$ $(t\to 0)$

STUDIES ON THE EFFECT OF CERTAIN DRUGS AND INSECTICIDES ON THE HEARTS OF INSECTS

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Introduction

The studies on the effect of drugs and insecticides on the isolated hearts of insects have not been made in much detail. Moreover uniform techniques have not been used by various workers. To quote a few Yeager *et al* (21) used modified Levy's solution, Yeager (18) ringer solution and Naidu (9) and Naidu and Zaheer (10) ringer solution with different constituents as perfusion fluid. Counting of heart beat have been made mostly under binocular microscope (9, 10, 19, 21) though electro cardiogram was the newest technique adopted by Uramoto (16) and Yeager (18). Kojhantchikov (7) used a lever which made contact with the heart by means of a piece of fat body tied to the free end of hair depending entirely upon the lever for magnification of heart movement and made a photographic tracing with a Kymograph camera. Uramoto (16) recorded the frequency of cardiac contraction by means of a light weight simple lever with one end of the outside of dorsal integument of silkworm larvae and other end recording on a Kymograph. Yeager (17) devised a most ingenious method of recording heart beat. The device consisted of a series of simple levers and an optical lever, the records being made by a shadow in beam of light which oscillated on a slit behind which a strip of sensitized paper is drawn. He criticized the techniques adopted by Kojhantchikov (7) and Uramoto (16) where magnification of fluctuation in heart beat was totally dependent upon levers. Uramoto's technique further seem to be faulty as it might have recorded the gut movement instead of cardiac movement (Patton, 12). Patton again comments over Yeager's device to be sensitive and successful but cumbersome set up with many inherent difficulties. The main basis of difference in perfusion fluid has been the constituents of blood and ease in handling and development in cardiac contraction recording techniques.

Numerous drugs and insecticides of different concentrations have been tried on isolated hearts of various insects. Yeager *et al* (21) studied the effect of 10 aliphatic thiocyanates on contraction rate and dilation on *Blatta orientalis*. Yeager *et al* (19) effect of nicotine concentrations on adult *Periplaneta*

This work formed part of the thesis of the junior author accepted for the Degree of Master of Science in Agriculture of the Agra University (1960) and was carried out under the guidance of the senior author.

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americana and *Prodenia eridania* larva. Yeager (17) effect of nicotine on amplitud and cardiac contraction of *Periplaneta americana*. Hamilton (6) effect of acetylcholine, atropine and nicotine on *Melanoplus differentialis*. Devenport (4) effect of nicotine atropine, curare, muscarine, adrenaline, acetylcholine and acetylbeta methyl-choline on *Stenopelmatus longispina*. Krijgsman *et al* (8) action of rotenone and TEPP on *Periplaneta americana* and Naidu (9) physiological action of acetylcholine chloride, adrenaline, non-adrenaline hydrochloride, ergotamine, eserine, pyrethrum, rotenone, pure parathion, paraoxon and p, p DDT and Naidu and Zaheer (10) site and mode of action of allethrin on *Periplaneta americana*. Yeager *et al* (20) studied the rate of contraction of isolated heart and malpighian tube of insects.

Recent studies of Naidu (9) and Naidu and Zaheer (10) were mainly intended to clarify the conflicting views on the presence of cholinergic and adrenergic systems in insects and also to investigate the physiological action of certain insecticides which have not been studied with reference to their site and mode of action.

It is clear from the above that studies on the effect of drugs and insecticides on insect hearts are fragmentary and not done in much details. It seems that there has been no work in India on this subject except that of Naidu and Zaheer (10)

It was only Yeager *et al* (19) who studied the comparative effect of nicotine concentrations on the hearts of adult *Periplaneta* and *Prodenia* larvae. Keeping all this in view present studies were taken up to elucidate the comparative effects of certain insecticides on isolated hearts of adults of *Mylabris phalerata* Poll and larvae of *Chilozonellus* Swinh. and *Dichocrocis punctiferalis* Guen. which are important crop pests.

Materials and Methods

Test insects

In the present studies, adults of *Mylabris phalerata* Poll. from groundnut, larvae of *Chilo zonellus* Swinhoe from Jwar and *Dichocrocis punctiferalis* Guen. from castor were collected from the Government Research Farm, Kanpur and used. Everytime fresh insects of uniform age and from the same food plants were collected to avoid probable variations in the results.

Physiological solution

Physiological perfusion fluid, viz. 1/7 Levy's stock solution employed by Yeager *et al* (19) was used on all isolated heart preparations, as they maintained a more or less constant rate of frequency and amplitude for more than 12 hours with this solution. The solution was prepared by adding Nacl 9 62 gms., Kcl-0 77 gm. and $CaCl_2$-0 50 gm. to one litre of water

Drugs and insecticides

50%, 25% and 95% wettable powders respectively of DDT parathion and ryania were used as insecticides and 40 nicotine sulphate as drug. Always fresh physiological and insecticide saline solutions were used for the experiments. Concentrations of substances in liquid form were used as v/v and those in solid form as w/v

Heart preparation

The specimen was placed in a wax block constantly bathed by the physiological solution. It was fixed with its dorsal surface down to the bottom of the wax cavity and the heart was clearly exposed

In the absence of running air in the laboratory a football with bladder was used. Compressed air from the bladder was bubbled through the physiological solution and thus a regulated amount of solution was dripping into the wax cavity at a constant rate of 105-120 drops per minute (25 drops=1 cc) The preparation was kept in a slanting position to let the solution drip down and not allowing it to accumulate in the cavity

After isolation, the hearts overcome the postoperative shock in about half an hour and then pulsated regularly for several hours. Therefore applications were made nearly half an hour after the dissection i.e. when heart beat was regular. Besides, every precaution was taken neither to disturb the contents of wax block nor to discontinue the flow of physiological solution over the heart preparation, which caused stimulation and retardation in heart beats. The temperature ranged between 29° 30 5°C.

The rate of pulsation was observed frequently with the help of binocular microscope, stop watch and counter. The observations were chiefly restricted to 4th and 5th abdominal segments though other heart regions were also observed. More frequent observations were taken just after the application of insecticidal solution. Apart from these, minor observations on amplitude peristalsis, diastolic pauses and form of contraction waves were also noted. Generally the period of observation lasted for 1½—4 hours though in some cases preparations were not discarded even after 8 or 9 hrs

The frequency of heart beat was obtained by determining time required for each 10 complete contractions and converting it into beats per minute (an integer) for which a conversion table was also prepared.

During each experiment an initial period of observation in physiological solution was devoted to control and several experiments with Mylabris and Chila were conducted in physiological solution to have some preliminary idea of heart beatings. Reversible action of insecticides, effect of drainage and fresh applications on a few larval hearts in situ were noted before they were transferred to wax blocks.

Dorsal blood vessels

The dorsal blood vessel of *Mylabris* consists of a dark brown, long narrow heart closed posteriorly and brownish thread like aorta which tapers anteriorly bifurcates apically and ends into the head. The heart is seven-chambered with one pair of lateral inlets (ostia) in each chamber The cardiac mechanisms of *Dichocrocis* and *Chilo* consist of twelve segmented heart and an aorta commencing from meta thorax and extending into the head. The heart of *Dichocrocis* is composed of blue black striated muscle fibrillae while that of *chilo* is hyaline. The pairs of alary muscles arise from the terga in case of *Mylabris* and inner dorsal body wall in larvae. They spread fanwise over the surface of dorsal diaphragm and fibres of one alary muscle meet beneath the heart with those of the opposite side of the body

Changes in frequency (f) of heart beat (hb) and gradient of stimulation (+) and depression (—) after application (appln.) of different concentrations (conc.) of nicotine (NIC) ryania (RYA) parathion (PAR) DDT or physiological solution (PHY SOL.) are shown in graphs. Arrows in the figures show the point of fresh application of solutions mentioned at their tail ends and each point in the graph generally indicates an average frequency of 5 countings each of 10 complete contractions.

EXPERIMENTAL

While conducting control experiments it was noted that exposed hearts generally attain regularity in half an hour time after dissection (Fig 1) When the solutions were drained, the frequency increased immediately which continued increasing till next application of physiological solution (Fig 6 broken line) Fresh transfers caused slight stimulation followed by depression in heart beat. The heart contraction rates were found to fluctuate around an observed hypothetical axis of normal rate of heart beat. The heart preparations exhibited a cycle of frequencies in ascending or descending order which reappears in the same order immediately after one is completed (Fig 2)

While studying larval hearts in situ there was also a great variation in pulse rates. Movements of larva caused stimulation while long rests followed retardation in heart beat frequency The frequency was much lower in isolated hearts than observed in hearts in situ (Fig 3) The depression was steep initially but gradual and irregular upto half an hour as mentioned.

The heart preparations showed pauses in diastole which occur frequently on prolonged exposures to saline solution. Subsequently after diastolic pause stimulated contraction was followed by depression again (Fig 2)

Hearts of *Mylabris* and *Chilo* showed a series of quick and delayed contractions. The longitudinal movements in *Mylabris* heart were easily seen to occur synchronically and asynchronically The longitudinal contraction alter

nated each transverse contraction, but 2-3 longitudinal movements also appeared between two systoles. In some experiments aorta showed fibrillation in between systole and diastole and it twisted towards right side in synchronisation with systole.

During periods of low pulse rates, the analysis of single heart beat was easy in *Mylabris* but to a lesser extent in larval hearts: systole (s), first diastole (d_1), second diastole (d_2) and diastasis (di) in former and first systole, second systole and diastole ($D = d_1 + d_2$) in latter heart preparations were sometimes detectable (Fig. 4). The average time lag of twelve observations while studying single heart beats in *Mylabris* heart showed following results:

	S to S = 2.0? Sec.	di + S = 0.67 Sec.
	$S + d_1 = 1.00$,,	di + d + di = 105 Sec.
	$d_1 + d_2 = 1.37$,,	
Therefore,	di = 0.13 Sec.	D = 1.37 Sec.
	$d_1 = 0.50$	S = 0.56
	$d_2 = 0.87$,,	

These periods may be placed in order as

$d_2 > S > d_1 > di$ and

$D > S > di$

Results

To study the action of nicotine sulphate and wettable powders of ryania, parathion and DDT on isolated hearts of *Mylabris*, *Chil.* and *Dichocrocis* following concentrations were maintained.

Mylabris

NIC—2×10^{-4} (A), 2×10^{-3} (B), 2×10^{-1} (C) and 8×10^{-4} (D)

RYA—475×10^{-4} and 95×10^{-4}

PAR—625×10^{-4} and 1875×10^{-4}

DDT—125×10^{-4} and 25×10^{-4}

Chilo

NIC— $2 \times 10^{-}$ and 8×10^{-4}

RYA—475×10^{-4} and 95×10^{-4}

PAR—625×10^{-4} and 1875×10^{-4}

DDT—$125 \times 10^{-}$ and 25×10^{-4}

Dichocrocis

NIC—2×10^{-4} RYA—5×10^{-3}

PAR—5×10^{-3} DDT—125×10^{-4}

Action of individual drugs and insecticides —

Nicotine on Mylabris hearts (Figs 5 6 and 7)

It is evident from graphs 5 6 and 7 that nicotine caused an immediate stimulation followed by depression at low conc. progressive depression followed by regular period of heart beat till it stopped in diastole at *intermediate* conc. and quick cessation at high conc. which continued till saline washings were made The gradients of stimulation and depression at 2×10^{-4} and 2×10^{-3} concentrations show little difference *during* stimulation but more steep depression occurred at lower concentration The application of low conc. of nicotine while the heart beat was in ascending order the gradient of stimulation was high followed by low gradient of depression and vice versa Prolonged exposure of heart to intermediate conc. produced much irregularity in heart beat and reverse peristalsis alternated with normal waves. *There* was much *reduction* in amplitude and fibrillation and twistings disappeared immediately after exposure to nicotine, the latter phenomenon reappeared after 20 mts. Besides, the heart preparations showed frequent diastolic pauses, uncoordinated heart beat and reduced amplitude with other nicotine concentrations also, specially when they were subjected to prolonged exposures. Only nicotine at 2×10^{-4} showed a regular heart beating for several hours. Draining of nicotine *solutions caused an increased* frequency and *amplitude* and *very clear* peristaltic and contraction movements in hearts.

The perfusion of nicotinized hearts A, B, C and D with physiological solution had marked effect and caused slight stimulation (unaltered hb) stimulation marked stimulation and complete revival of hearts respectively The delayed effect of saline washing showed a regular heart beat at lowest conc. but with other concentrations heart attained the same heart beat in all the three cases. The stimulated hearts showed gradual fall or unaltered heart-beat when subjected to saline washing the former being associated with short and latter with prolonged *exposure* to nicotine solution

Nicotine on larval hearts (Figs 7 and 8)

It is apparent from graphs 7 and 8 that NIC 2×10^{-4} on *Dichocrocis* and NIC 2×10^{-4} on *Chilo* hearts had similar action as that of all the three concentrations on *Mylabris* hearts. Two applications of NIC 2×10^{-3} on *Dichocrocis* heart show little difference in the gradient of stimulation but a high gradient of depression on first application which is equivalent to the action of two lower concentrations of nicotine on *Mylabris* Similarly regular heart-beat, frequent diastolic pauses and cessation at 2×10^{-3} conc. coincide with the action of same concentrations on *Mylabris* heart Larval hearts also showed *reduced* amplitude and more frequent and prolonged diastolic pauses even *after* repeated washing with physiological solution

The perfusion of *Dichocrocis* heart with physiological solution had *an* immediate revival in heart beat and the gradient of stimulation was equal in both

Dichocrocis and *Mylabris*. However *Chilo* heart revived late at 2×10^{-3} and not at all at 8×10^{-3} nicotine concentration. The revived heart of *Chilo* showed irregular and slow heart beat with frequent diastolic pauses after each diastole. Finally the heart stopped in diastole within 2 hours.

Prolonged action of nicotine (Fig. 8)

It is clear from Fig. 8 that application of perfusion fluid and nicotine both stimulate the heart beat but alternate washing with physiological and nicotine solutions caused retardation in the stimulation of heart beat frequency on each successive occasion. Two successive nicotine treatments had paralysing instead of stimulating effect. It is also evident that gradient of stimulation was high at higher nicotine conc. or 2nd application of nicotine or saline solution, but gradient of depression was high at low nicotine conc. or 1st nicotine or saline application. Delayed effect of such perfusion showed a progressive regularity in heart-beat frequency.

Ryania (Fig. 9)

The treatment of *Dichocrocis* heart with RYA 5×10^{-4} and *Chilo* and *Mylabris* hearts with RYA 475×10^{-5} and 95×10^{-4} showed a specific immediate fibrillation and cessation of all the five hearts in diastole. In addition, *Mylabris* heart also showed twistings and systolic arrest initially at RYA 95×10^{-4}. The former phenomenon merged into simple linear movement after 7 mts. while systoled heart gradually expanded to restore a semi-systolic or diastolic standstill within 5 mts. But *Mylabris* hearts treated with RYA 475×10^{-4} started pulsating within 8 minutes. The movement and pulsation mentioned were noted only in thoracic and 2nd abdominal segments respectively. Besides these, irregular heart beat and alternate fibrillation after each 2 or 3 systoles were also noted.

Saline perfusion of *Mylabris* and *Chilo* hearts after 40 and 60 minutes treatment with RYA 475×10^{-4} and *Dichocrocis* heart after 30 minutes treatment with RYA 5×10^{-4} showed revival of latter heart only within 2.3 mts. Later the revived heart exhibited diastolic pauses and uncoordinated heart beat.

Parathion (Figs. 10, 11)

Parathion 5×10^{-4} on *Dichocrocis* and PAR 1875×10^{-6} on *Chilo* and *Mylabris* hearts had no quick action but PAR 625×10^{-6} had a stimulating action on *Mylabris* and *Chilo* hearts. The gradient of change in heart beat shows no immediate effect on *Dichocrocis* heart with PAR $5 \times 10^{-}$. There is more steep stimulation to *Chilo* heart with PAR $625 \times 10^{-}$ but more steep depression to *Mylabris* with PAR $1875 \times 10^{-}$.

The subsequent effect of all the concentrations of parathion on 3 insects hearts was quick and followed by gradual depression and cessation of heart

The cause of reduced amplitude has been explained by Yeager *et al* (17) to be the inhibitory action of nicotine on heart muscles but it fails to explain the findings of the present work. When the nicotine solutions were drained away there was an increase in frequency though much of the solution remain adhered to perfuse the heart. Reduction in amplitude have been reported in other insect hearts on application of nicotine (6 7 19) which shows the existence of this property in the drug itself and not in insect heart

Yeager *et al* (19) reported a correlation between a faster rate of heart beat and decreased amplitude. The cause he suggested was faster rate, which reduces the amplitude. But the suggestion fails to explain why Hamilton (6) found reduced amplitude though heart exhibited unaltered heart beat and why gradual depression does not associate with gradual increase in amplitude. The probable answer would be that stimulation and depression occurs due to action of nicotine on ganglia but reduced amplitude is due to its action on muscle fibrillae and when stimulation and reduction in amplitude occur simultaneously we confuse to call it a correlation

Ryania

In case of ryania the results indicate that it is equally rapid in effect and seemingly has the same site of action as that of nicotine. Immediate diastolic cessation of heart at almost equal or higher concentration of ryania, appearance of fibrillation and twistings in thoracic heart segments at 95×10^{-4} conc and uncoordinated heart beat lend further support to the above fact.

Pulsation of *Mylabris* heart at 475×10^{-5} conc. after 8 minutes exposure but not of *Chilo* at the same conc. and of *Dichocrocis* at still lower conc. (5×10^{-4}) further justify the idea that adults take more time than the larvae to produce the same symptoms to any insecticide. Consequently it further supports that both larval hearts are reacted upon in the same way with both the insecticides.

Revival of *Dichocrocis* at low conc and non-revival of other hearts subjected to high conc show the inhibitory action of ryania in direct relation with concentration. Similar results were obtained with nicotine It still more emphasizes that ryania and nicotine are almost similar so far as paralysis of hearts and site of action are concerned. The hearts, however showed a more specific fibrillation with ryania than with nicotine

Parathion

The similarity in appearance of symptoms of paralysis, revival and stimulation at parathion 625×10^{-4} and delayed appearance of paralysing symptoms at 1875×10^{-4} conc in *Mylabris* and *Chilo* hearts leads us to believe that parathion has the same site of action in both adults and larvae However no immediate action on *Dichocrocis* heart followed by quick depression and absence of gradual depression from the series of symptoms of paralysis at PAR 5×10^{-4} and its revival non-revival of *Chilo* at 625×10^{-4} conc. and revival of the same at

1875×10^{-4} conc. and revival of *Mylabris* heart at 625×10^{-4} and not at 1875×10^{-4} conc., make any definite conclusion difficult and need further elucidation. It seems that *Dichocrocis* heart posses some special mechanism which initially checks the stimulation in heart-beat at low conc but no longer resists the action of parathion at high conc. or long exposure at low conc.

The findings of the present study are in agreement with those of Orser and Brown (11) and Brown (3) They have observed stimulation in pulse rate of *Periplaneta* with a final systolic arrest on injection of 80 micrograms of parathion both in vivo and vitro Naidu (9) reported that pure parathion had no effect on heart beat but exposure to ultra-violet light had an anti cholinesterase activity He pointed out that parathion did not show such property during experiments though some workers (1 5 13) reported it to be a powerful inhibitor of cholinesterase in vivo He has pointed out to the possibilities that parathion employed by authors for testing might have been contaminated with paraoxon or di-ethyl S-p- nitrophenyl thiophosphate which possesses anticholinesterase activity in vitro. He further justified the statement by showing stimulating action of paraoxon on *Periplaneta* Evidently it indicates that stimulating and inhibiting action of parathion wettable powder used during the present work is due to its contammination with other isomers of parathion. But the delayed appearance of paralytic symptoms at high conc. and stimulatory ones followed by inhibitory action at low conc. make any conclusion difficult. It seems that two different constituents act together on the heart, one of which stimulates while other inhibits the pulsation. Stimulation may be due to paraoxon (9) but what inhibits the heart beat, remains to be answered. The answer may be S-p isomer of parathion which has also insecticidal action (2) Stimulation in heart beat at low conc. and delayed appearance of paralysing symptoms at high conc. of parathion was probably due to constant stimulating action of paraoxon and increasing inhibiting action with increase in concentration of S-p isomer

DDT

The findings of the present work allude to the fact that two larvae have similar initial response to DDT concentrations but adults are more resistant and require higher inhibitory concentration to exhibit the same response. Immediate revival of *Mylabris* heart, gradual revival of *Dichocrocis* Heart and non-revival of *chilo* heart are presumably due to increasing acute response of three hearts. Larval hearts seem to respond more acutely than adult hearts.

Revival of *Mylabris* and *Dichocrocis* hearts to the same degree and their regular pulsation even after 6¼ hours and 9¼ hours' saline perfusion further prove that insecticides have much less toxic action on two hearts than that on *Chilo*. Stimulated initial effect of saline perfusion shows stimulating action of lower lethal dose of DDT on heart muscles.

Naidu (9) reported a very erratic effect of DDT 10^{-4} which is probably due to very low conc. used by the author in comparison to the concentration used in the present studies. However present findings totally disagree with the findings of other investigators (3 4 15)

SUMMARY

The action of different concentrations of nicotine sulphate, ryania, parathion and DDT on the frequency of heart beat of isolated heart preparations of adult *Mylabris phalerata* and *Chilo zonellus* and *Dichocrocis punctiferalis* larvae, immersed in an aerated physiological solution have been studied.

Application of nicotine 2×10^{-4} and 2×10^{-4} on *Mylabris* and NIC 2×10^{-4} on *Dichocrocis* heart initially stimulated the frequency but NIC 2×10^{-4} and 8×10^{-4} caused progressive depression and cessation respectively both in *Mylabris* and *Chilo* hearts. Thus higher the concentration quicker the response. The adult heart responds rather slowly

Ryania 5×10^{-4} on *Dichocrocis* 475×10^{-4} and 95×10^{-4} on *Chilo* and *Mylabris* hearts immediately arrested the heart in diastole. However *Mylabris* heart was pulsating 8 minutes after treatment with RYA 475×10^{-4} which again stopped gradually Besides, the *Mylabris* heart treated with RYA 95×10^{-4} showed fibrillation twistings and uncoordinated heart beat in thoracic and 2nd abdominal region.

Mylabris and *Chilo* hearts showed stimulation at parathion 625×10^{-4} while PAR 1875×10^{-4} had a gradual depression followed by cessation. *Dichocrocis* heart remained initially unaffected followed by cessation at PAR 5×10^{-4}

DDT at 125×10^{-4} had stimulating action followed by depression in case of *Mylabris* but it showed immediate inhibitory action on *Chilo* and *Dichocrocis* hearts. Further DDT 25×10^{-4} immediately stopped the *Mylabris* and *Chilo* hearts. The paralyzing action of DDT has been found reversible after repeated washing with physiological solution.

ACKNOWLEDGEMENTS

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References

1. Aldridge W. N. and Barnes, J. M. 1952. Some problems in assessing the toxicity of the "organo-phosphorus" insecticides towards mammals. *Nature* 169 : 345

2. Bayer 1951 German Pat. : 617 33

3. Brown, A. W. A., 1951. Insect control by chemicals: 525. New York. Wiley; London, Chapman and Hall.

4. Davenport, D. 1949. Studies on the pharmacology of the heart of orthopteron *stenopelmatus*. *Physiol. Zool.*, 22 : 35

5. Diggle W. M. and Gage J. C., 1951. Cholinesterase inhibition by parathion in vivo. *Nature* 168 : 998.

6. Hamilton H. L., 1939. Action of acetylcholine, atropine and nicotine on the heart of grasshopper (*Melanoplus differentialis*). *J. Cell Comp. Physiol.* 13 : 91

7. Kojhantchikov I. V. 1932. The normal pulsation frequency of Dorsal vessel on *Blatta orientalis* L. and the effect of certain factors on it. Leningrad Inst. control Farm and Forest pests, Bull. 2 : 149-172, Illus. (In Russian summary in English, PP. 171-172, translated by Korsa Koff)

8. Krijgsman, B. J. Dresden, D. and Berger N. E., 1950. The action of rotenone and TEPP on the isolated heart of *Periplaneta*. *Bull. ent. Res.* 41 : 141

9. Naidu, M. B., 1955. Physiological action of drugs and insecticides on insects. *Bull. Ent. Res.* 46 (1) : 220. Illus.

10. Naidu M. B. and Zaheer S. H., 1956. Physiological action of allethrin on insects. *Indian J. Ent.*, 18 (1) : 57-62. Illus.

11. Orser W. B. and Brown, A. W. A., 1951. The effect of insecticides on the heart-beat of *Periplaneta*. *Canad. J. Zool.* 29 : 54

12. Patton, R. L. 1953. Study on the circulatory system in insects. I. Shepard' "Methods of testing chemicals on insects. I : 61

13. Payton, J. 1953. "Parathion and ultra violet light. *Nature* 171 : 355.

14. Prosser C. L., 1940. Action potentials in the nervous system of the crayfish. Effect of drugs and salts upon synaptic transmission. *J. Cell Comp. Physio.* 16 : 25

15. *Tobias, J. M. Kollros, J. J. and Savit, J. 1946. Acetylcholine and related substances in the cockroach, fly and crayfish and the effect of DDT. *J. Cll. Comp. Physiol.* 28 : 159

16. Uramoto, S. 1932. On the mechanogram and electrogram of the dorsal vessel and alimentary canal in the silkworm larva. *Bull. Imp. Seric. Expt. Sta.* (*Tokyo*) 8 : 121-134. Illus. (I. Japanese, English resume, p. 134)

17. Yeager J. F. 1938. Mechanocardiographic methods of recording insect heart activity with reference to effect of nicotine on isolated heart preparation of *Periplaneta americana*. *Jour. Agr. Res.* 56 : 267-276

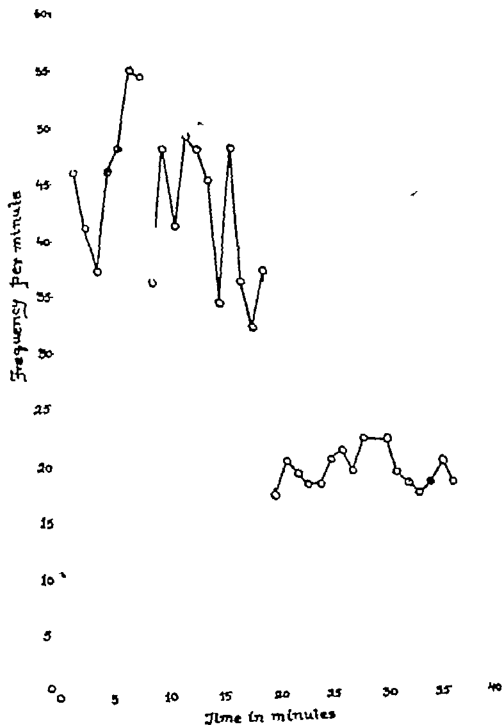
18. Yeager J. F. 1938. Electrical stimulation of isolated heart preparations. *Jour. Agr. Res.* 59 : 121-138. Illus.

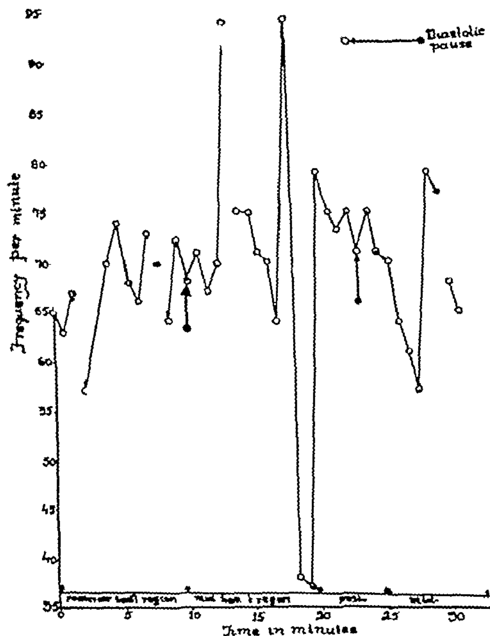
19. Yeager J. F. and Gahan, J. B. 1937. Nicotine action on heart of roach and southern armyworm. *Jour. Agr. Res.* 55 (1) : 1-19. illus.

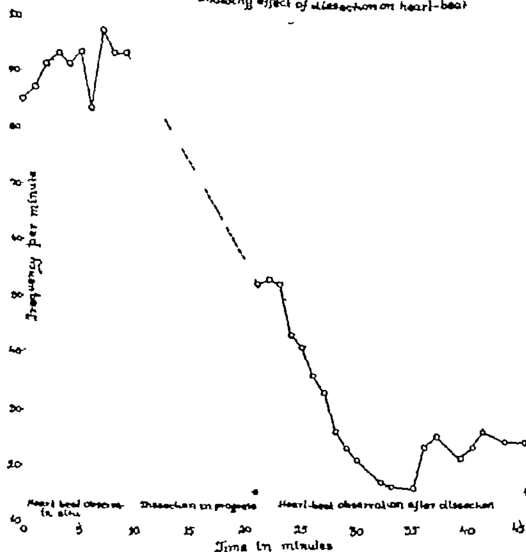
20. Yeager J. F. and Hager A. 1934. On the rate of concentration of isolated heart and malpighian tube of insect, *Periplaneta orientalis*, Method. *Iowa state Coll. Jour. Sc.* 8 : 391-95. illus.

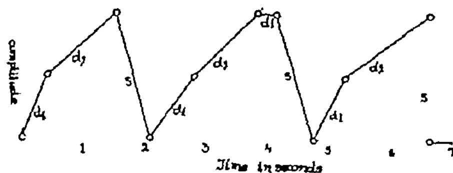
21. Yeager J. F. Hager A. and Straley J. M., 1935. Action of thiocyanates on heart, roach. *Ann. Ent. Soc. Amer.* 28 : 256-264

* Only abstract has been consulted.


Fig no 1
Chilo heart in physiological solution (control)
showing changes in frequency in relation to time lasted after dissection
Frequency per minute
Time in minutes
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70


Fig. no. 2
Chilo heart in physiological solution
Showing cycles in heart beat frequency
Diastolic pause
Frequency per minute
100
95
90
85
80
75
70
65
60
55
50
45
40
35
0
5
10
15
20
25
30
Time in minutes


Fig no 3
Chilo heart in physiological solution
Showing effect of dissection on heart-beat
Frequency per minute
Heart beat observation in situ
Dissection in progress
Heart-beat observation after dissection
Time in minutes
0
5
10
15
20
25
30
35
40
45
20
30
40
50
60
70
80
90



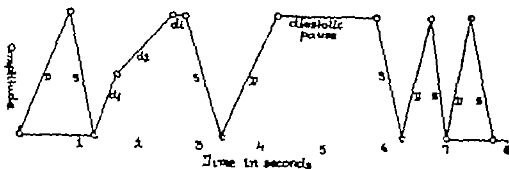
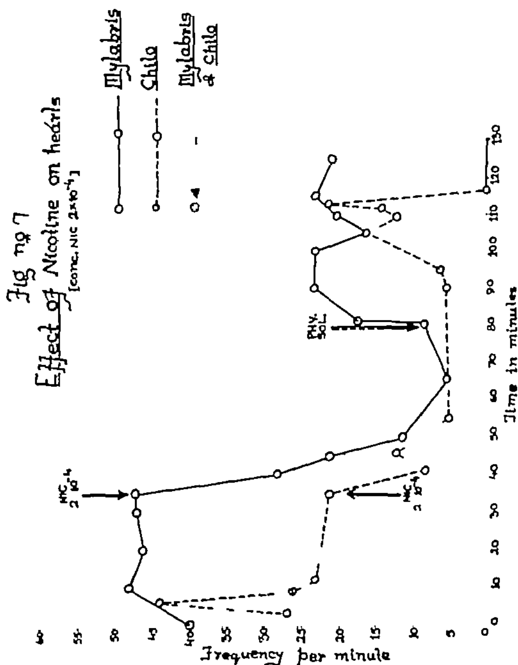
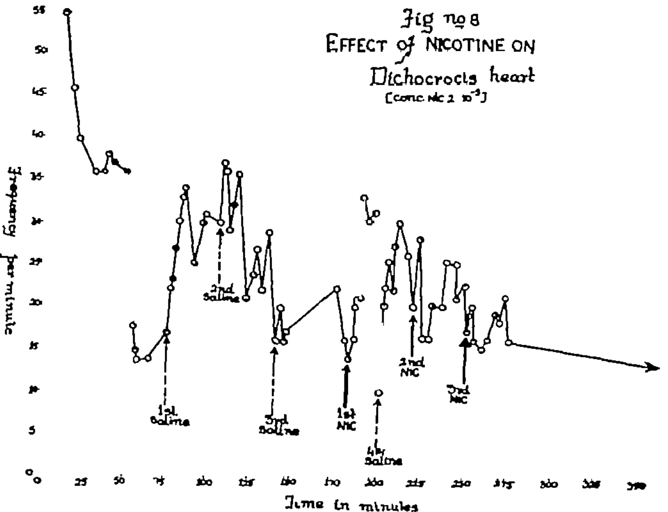


Fig. No. 4 Analysis of single heart beats in Mylabris
Heart in physiological solution (above)
Nicotinized heart (below)


Fig no 7
Effect of Nicotine on heart
Mylabris
Chilo
Mylabris & Chilo
NIC 2 10^-4
PHY. SOL
Time in minutes
Frequency per minute


Fig no 8
EFFECT OF NICOTINE ON
Dichocrocis heart
1st Saline
2nd Saline
3rd Saline
4th Saline
1st NIC
2nd NIC
3rd NIC
Frequency per minute
Time in minutes

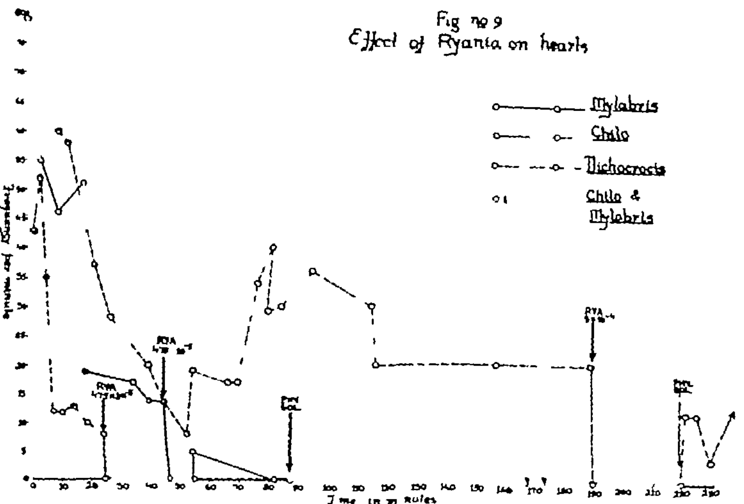
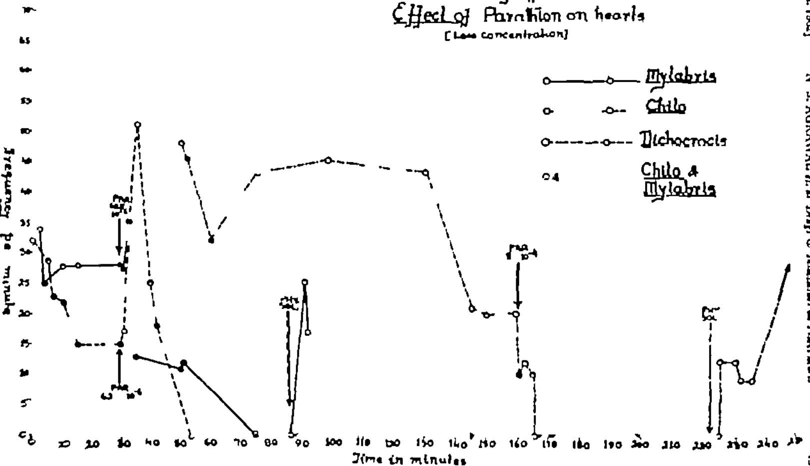
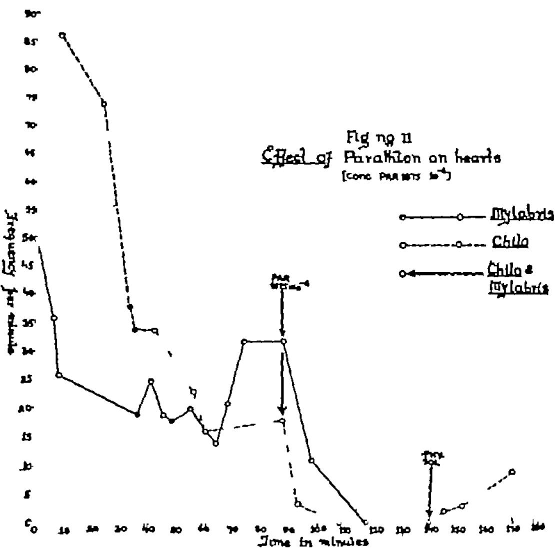
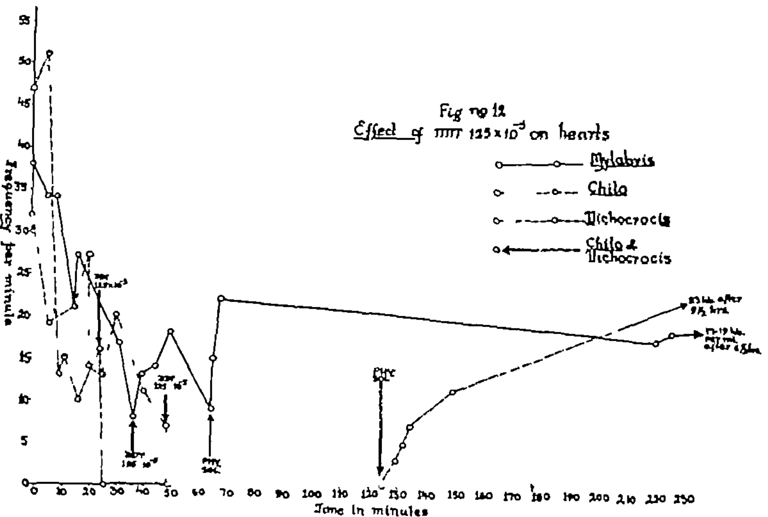


Fig no 9
Effect of Ryania on hearts
Mylabris
Chilo
Dichocrocis
Chilo & Mylabris
Frequency per minute
Time in minutes


Fig no 10
Effect of Parathion on hearts
(Less concentration)
Mylabris
Chilo
Dichocrocis
Chilo & Mylabris
Time in minutes
Frequency per minute


Fig no 11
Effect of Parathion on hearts
Mylabris
Chilo
Chilo & Mylabris
Time in minutes


Fig no 12
Mylabris
Chilo
Chilo &
Time in minutes
Frequency per minute

THE MORPHOLOGY OF *WALLAGO ATTU* (BL. & SCHN.)
[The Air Bladder and Weberian Ossicles]

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Weber (1920) was the first to point out that in certain groups of physostomous fishes a connection by movably interconnected ossicles exists between the auditory organs and the air bladder To commemorate the memory of this pioneer worker the ossicles have subsequently been called the Weberian ossicles. Segmehl (1885) coined the name Ostariophysi for the group of fishes having such a connection and Bridge & Haddon (1889 1892 & 1893) gave a detailed account of the air bladder and Weberian ossicles in the family Siluridae Hora (1937), Nair (1937 & 1938) and Dobbin (1941) described the air bladder in a number of Siluroid fishes and Jones & Marshall (1953) reviewed the position with regard to the structure and functions of the teleostean air bladder Bedault (1868) Nusbaum (1881 & 1908) Grassi (1881) Wright (1884) Sorensen (1884) Segmehl (1885 & 1891) Bridge & Haddon (1893) Goodrich (1909) Kindred (1919) Hora (1922) Matveiv (1929) and Watson (1939) studied the structure and homologies of the Weberian ossicles in a number of fishes.

The Air Bladder

The air bladder lies above the alimentary canal and extends from the posterior limit of the gill cavities to about two-third the length of the body cavity behind. It is closely adpressed to the wall of the body cavity on all sides except on the ventral, where it is free. Applied to its anterior end is the head kidney and at its posterior end lies the posterior part of kidney

The air bladder (Fig 1) is heart-shaped, convex on the dorsal side and more or less flat on the ventral. Along its mid-dorsal line there is a longitudinal groove for the vertebral column, which is more prominent in the anterior one third for reception of the ventral keel of the complex vertebra. The association between the air bladder and the ventral keel is so intimate that the bladder ruptures along this contact in an attempt to separate it from the vertebral column. From the ventral side of the bladder about one-fifth its length from the anterior end arises the non-osseous sigmoid pneumatic duct. It is one-third the length of the air bladder and runs forward to the right to open into the oesophagus. Arising from the roof of the bladder at one third the length from the anterior end and at the level of the fifth vertebra is an antero-posteriorely compressed ligamentous pillar which runs down to the floor slightly inclined forward. The pillar is twice as long as it is broad. Its anterior face is slightly grooved and at its insertion on the floor of the bladder in the groove opens the pneumatic duct. From the posterior face of the pillar arises a vertical septum, which runs

to the posterior end of the bladder The pillar and the longitudinal septum divide the cavity of the bladder into the anterior Weberian chamber of Charanllov and two postero-lateral chambers. The anterior chamber occupies the one-third of the bladder and communicates widely with the postero-lateral chambers on either side of the pillar

The bladder consists of two main layers, an outer thick and rigid tunica externa and an inner thin and membranous tunica interna. Its ventral face is covered by peritoneum.

The air bladder is held in position partly by close association of its anterior one third to the vertebral column and partly by the anterior and posterior ligamentous bands. The anterior bands arise from the front end of the bladder and run backwards underneath the vertebral column to the radial nodules below the complex vertebra. The posterior bands develop from the dorsal side of the air bladder about the level of the fifth vertebra. Each band separates into two strips, one of which gets attached to the side of the fifth vertebra and the other runs forward enclosing the lateral ossification and ends on the ventral ridge of the tripus of its side. The air bladder is further secured by the anterior divisions of the parapophyses of the fourth vertebra. By its front edge each division curves down along its side of the bladder and marks its course by a shallow groove on its surface.

Behind the pectoral girdle and dorsal to the origin of the pectoral fin, the external surface of the fish is marked on either side by a triangular lateral cutaneous area. Each lies with its base immediately behind the girdle and the apex directed backwards. It is half as long as the pectoral fin and at its base half as wide as it is long The area is bereft of musculature and arises by the divergence of dorso-lateral and ventro-lateral muscles upwards and downwards respectively Around the lateral cutaneous area a wider region can be made out, where the musculature is thinner in comparison to the rest of the body Over the lateral cutaneous areas abut the antero-lateral ends of the air bladder

The primary function of the air bladder is hydrostatic and it assists the fish in maintaining a certain position in the water In a fish with bladder very little fin movement is needed to keep it at a particular depth. The pressure in the air bladder is maintained by secretion and absorption of the gas by blood. In physostomous fishes the secretion and absorption is said to be supplemented or mostly done by taking in and throwing out of the air through the pneumatic duct. The secretion is due to the increase in the hydrogen ion concentration in the air bladder glands by external stimulus.

The Weberian Ossicles

The Weberian ossicles (Figs. 2 & 3) consist of a pair of ossicle-chains one on either side of the vertebral column, which connect the internal ear with the anterior chamber of air bladder Each chain is composed of four bony elements—the claustrum, scaphium, intercalarium and tripus aligned anteroposteriorely

The claustrum, scaphium and intercalarium are very delicate while the tripus is well developed.

The *claustrum* is embedded on its side in the fibrous wall above the exoccipital. It is slender and spine-like curved at its lower end and pointed at the upper. Its lower end lies on the outer side of the scaphium in the angle between its ascending and the spatulate processes and the upper end immediately behind the neural plate of the exoccipital.

The *scaphium* lies just behind the claustrum. It consists of an ascending and a horizontal process united at right angles. The ascending process is embedded in the fibrous wall of neural canal parallel to and behind the claustrum. It is poorly developed and is in the form of a curved spicule with a finely pointed upper end. The horizontal process is comparatively prominent and forms the main part of the ossicle, which passes forward from the lower end of the ascending process. A spoon-like expansion at its front end closes the external atrial aperture at the hind end of the exoccipital. At the junction of the ascending and the horizontal processes on the inner side is a spherical nodule, which moves in a socket along its side of the posterior end of the basioccipital bone.

The *intercalarium* is reduced to a nodule of bone, which lies in the interossicular ligament and covers its full width. The interossicular ligament is short and extends from the outer side of the horizontal process of the scaphium to the anterior extremity of the tripus.

The *tripus* is the largest ossicle of the series and lies on the outer side of the exoccipital and the first and complex vertebrae. It is dorso-ventrally flattened and is divided into two parts, an anterior and a posterior. The anterior part runs along the outer side of the exoccipital, first vertebra and anterior one third of complex vertebra. Its external margin is slightly convex and the internal slightly concave. The posterior part or the crescentic process is horse shoe-shaped and it easily breaks off from the rest of the ossicle. The part lies with its concavity directed inwards along the outer side of the rest of complex vertebra in the tunica externa of the anterior chamber of air bladder. From the inner side of the ossicle at about the junction of the anterior and posterior parts is given off a triangular articular process, which articulates on the complex vertebra. Along the ventral side of the tripus is the obliquely directed ventral ridge running from the posterior part to the articular process.

The anterior part and articular process of the tripus, the intercalarium and the part of interossicular ligament, in between the tripus and intercalarium are contained in the saccus paravertebralis of Weber which is a thin walled fibrous sac filled with a colourless fluid. The sac is paired and each extends on its side along the lateral aspect of the exoccipital, first vertebra and anterior one-third of the complex vertebra from the external atrial aperture as far back as to the insertion of the posterior part of the tripus in the air bladder

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The primary function of the air bladder is hydrostatic and it assists the fish in maintaining a certain position in the water In a fish with bladder very little fin movement is needed to keep it at a particular depth The pressure in the air bladder is maintained by secretion and absorption of the gas by blood. In physostomous fishes the secretion and absorption is said to be supplemented or mostly done by taking in and throwing out of the air through the pneumatic duct. The secretion is due to the increase in the hydrogen ion concentration in the air bladder glands by external stimulus.

The Weberian Ossicles

The Weberian ossicles (Figs. 2 & 3) consist of a pair of ossicle-chains one on either side of the vertebral column which connect the internal ear with the anterior chamber of air bladder Each chain is composed of four bony elements—the claustrum, scaphium, intercalarium and tripus aligned anteroposteriorely

Workers	Claustrum	Scaphium	Intercalarium	Tripus
11 Kindred (1919)	Intercalated arch	Neural arch of first vertebra	Neural arch of second vertebra	Rib of third vertebra
12. Hora (1922)	Neural arch of first vertebra	Part of neural arch of second vertebra	Neural arch of second vertebra	Transverse process and rib of third vertebra and rib of fourth vertebra
13 Matveiv (1929)	Neural arch of first vertebra	Neural arch of first vertebra	Neural arch of second vertebra	Rib of third vertebra.
14 Watson (1939)	Direct ossification from wall of atria sinus imparis	Part of neural arch of first vertebra	Part of neural arch of second vertebra	Transverse process of third vertebra

SUMMARY

The air bladder and Weberian ossicles of the fish have been described in necessary details and a table on the homologies of the ossicles has been provided.

1 The air bladder is heart-shaped and its cavity is divided by a T-shaped arrangement of a transverse and a longitudinal septum into an anterior and two posterior chambers. Owing to the transverse septum being incomplete the two posterior chambers communicate widely with the anterior chamber

2 The lateral cutaneous areas, along which the bladder is in contact with the outer skin, are triangular and lie above the pectoral fins.

3 The bladder is held in position by close association with the anterior one-third of the vertebral column, by the anterior divisions of transverse processes of fourth vertebra and by the bands. The anterior bands connect the front end of the bladder with the radial nodules. Each of the posterior bands separates into two strips, one gets attached to the fifth vertebra and the other running forward ends on the ventral ridge of tripus.

4 Among the Weberian ossicles the claustrum is a slender curved spicule. The scaphium comprises of a spicular ascending process and a well developed horizontal process. The spoon-like expansion at the front end of horizontal process closes the external atrial aperture, while the nodule at its junction with the ascending process articulates in a socket on the basioccipital.

5 The intercalarium is merely a nodule in the interossicular ligament. The tripus is the most prominent ossicle. It is distinguished into the anterior and posterior parts, which easily break off. From the junction of the two

parts arises the articular process, which articulates on the complex vertebra. On the ventral side of the ossicle is an obliquely directed ventral ridge

6 The anterior part and articular process of tripus, the interclarium and the part of interossicular ligament between the tripus and interclarium lie in the saccus paravertebralis, which is filled with a colourless fluid.

References

1 Bridge T W & Haddon, A. C. 1889 Contributions to the anatomy of fishes. I. The air bladder and Weberian ossicles of Siluroidae. *Proc Roy. Soc London* XLVI 'B 309-328

2. Bridge T W & Haddon, A. C. 1892 Contributions to the anatomy of fishes. II The air bladder and Weberian ossicles in Siluroid fishes. *Proc Roy Soc London* LII 'B' 139-157

3 *Bridge T W & Haddon, A. C. 1893 Contributions to the anatomy of fishes.* The air bladder and Weberian ossicles in Siluroid fishes. *Phil. Trans Roy. Soc London* CLXXXIV 'B' 65-334

4 De Beer G R. 1937 The development of the vertebrate skull. pp. 136-141

5 Dobbin, C 1941 A comparative study of the gross anatomy of the air bladder of ten families of fishes of New York and other eastern states. *Jour Morph.* LXVIII 1-29

6. Goodrich, E. S 1930. Studies on the structure and development of vertebrates. pp. 578-591

7 Hall, F G 1924 The functions of the swim bladder of fishes. *Biol Bull. Woods Hole* XLVII: 79-114

8 Hora, S L. 1922. The homologies of the Weberian ossicles. *Jour Asiat. Soc* Bengal. XVIII 1-4

9 Hora, S L. 1937 Correlation between the disposition of the liver and the kidney and the form of the air bladder in certain Siluroid fishes of India. *Proc Nat Inst. Sci. India.* III 31-43

10. Nair K. K. 1937 Changes in the internal structure of the air bladder of *Pa gasius pangasius* during growth. *Rec Ind. Mus* XXXIX 117 124

11 Nair K. K 1938. Changes in the internal structure of the air bladder of *Silonia silondia* during growth. *Rec. Ind Mus* XL 5-11

12 Nair K. K.1938 Changes in the internal structure of the air bladder of *Eutropiichthys vacha*, *Clupisoma garua* and *Ailia coila* during growth *Rec Ind. Mus.* XL 185-187

13. Nelson, E. M. 1948 The comparative morphology of the Weberian apparatus of the Catostomidae and its significance in systematics *Jour Morph.* LXXXIII 225-252.

14 Jones, F R., Harden & Marshall, N B 1953. The structure and functions of the teleostean swimbladder *Biol. Revs. Cambridge Phil Soc* XXVIII: 16-83.

15. Nelson, E. M. 1949 Swim bladder and Weberian apparatus of *Rhamdia sulphurea* *Jour Morph.* LXXXIV 495-524

16. Sarbahi, D S 1932 The endoskeleton of *Labeo rohita*. *Jour Roy Asiat Soc Bengal N S.* XXVIII 295-347

17 Watson, J M. 1939 The development of the Weberian ossicles and anterior vertebrae in the goldfish. *Proc Roy Soc London.* CXXVII 'B 452-472

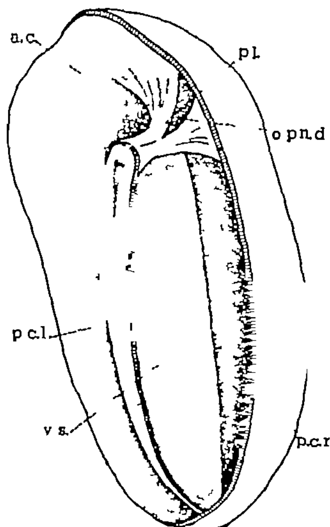


Fig. 1 The Air Bladder

a. c., anterior chamber *o. pn. d.* opening of penumatic duct, *pl.*, pillar; *p. c. l.*, posterior chamber lef *p. c.* posterior chamber right *v. s.* ertical septum

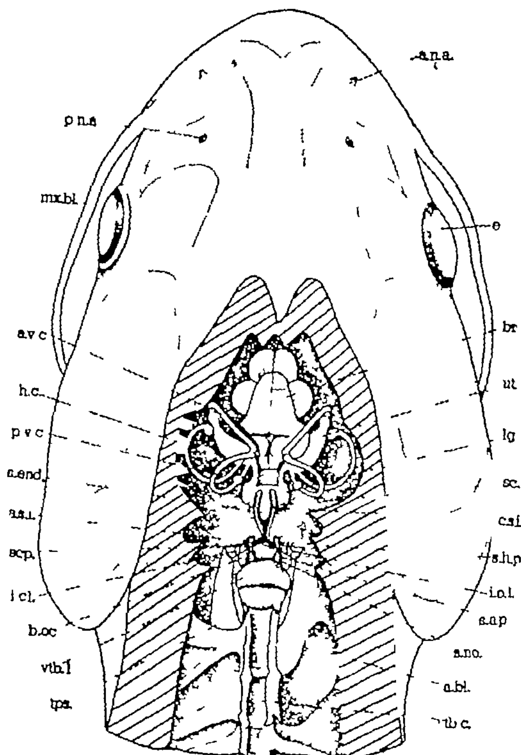


Fig. 2 Dorsal view of the brain, ear and Weberian ossicles

a.n.a. anterior narial aperture; *a.bl.*, air bladder *a.s.i.* atrium sinus imparis; *a.v.c.* anterior vertical canal *b.oc.*, basioccipital *br.*, brain; *c.s.i.*, cavum sinus imparis *e* eye;

h.c. horizontal canal *i.cl.* interclarium *i.o.l.*, interossicular ligament *lg.* lagena; *mx.bl.*, maxillary barbel *p.n.a.* posterior narial aperture *p.v.c.*, posterior vertical canal *sph.* scaphium *s.* sacculus *s.a.p.* scaphium ascending process *s.h.p.* scaphium horizontal process; *s.no.*, scaphium nodule; *s.end.* sinus endolymphaticus *tp.*, tripus *ut.* utriculus; *v.th.* 1 first vertebra *c.th.c.*, complex vertebra.

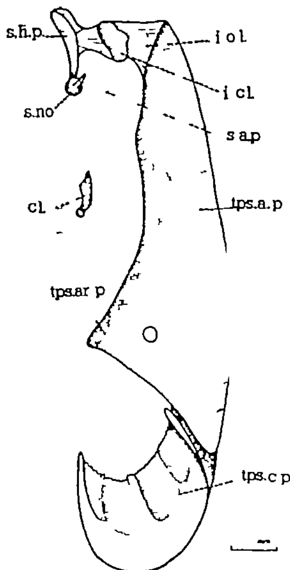


Fig. 3. The Weberian Ossicles

cl., claustrum; *i.cl.* interclarium *i.o.l.*, interossicular ligament *s.a.p.* scaphium ascending process; *s.h.p.*, scaphium horizontal process; *s.no.* scaphium nodule *tps.a.p.*, tripus anterior part; *tps.ar.p.*, tripus articular process; *tps.c.p.*, tripus posterior part.

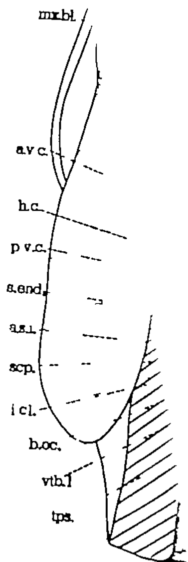


Fig. 2 Dorsal vi-
a.n.a. anterior narial aperture;
anterior vertical canal; *b.oc.*, basi-

MORPHOLOGICAL STUDIES IN CYPEARACEAE

1 Development of the Ovule and the Gametophytes In *Fimbristylis dichotoma* Vahl

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Introduction

Embryological studies in the Cyperaceae are far from adequate and what ever work has been done is chiefly confined to the microsporogenesis because of its unusual course. Work done on the embryology of the family upto 1930 has been well reviewed by Schnarf in 1931 After that Tanaka has published a number of papers (1937 38 39 40 and 41) in which he has cheifly dealt with the cytological aspects of microsporogenesis. Dnyansagar & Tiwari (1956a, b) have recently published two small papers on the sporogenesis and gametophytes of *Fimbristylis quinquangularis* The embryology of *Cyperus rotundus* and *Cyperus tegetum* Roxb has also been worked out by Khanna (1956) and Padhye and Moharir (1958) respectively

Material and Method

The material was collected during the months of September October and early November in 1956 from Jaipur Ajmer and localities round about Agra. It was fixed in Formalin-acetic-alcohol and HF treatment was given for about 8 days prior to dehydration Sections were cut at 6 to 12 microns and stained with (1) Safranin Fastgreen, (2) Haematoxylin-Orange G and (3) Haematoxylin-Erythrosin.

Flower

The flowers are grouped into spikelets which are about 2 5 mm to 3 75 mm in length when young but reach a length of 7 5 mm after the fall of the lower glumes. They are arranged in subcompound umbels. In an umbel there is a sessile spikelet, 2 or 3 spikelets on pedicels of different lengths and 2 or 3 rays which bear one sessile spikelet and one or two pedicelled spikelets. The spike lets are many flowered. Each flower develops in the axil of a glume and consists of a stamen and a carpel (Fig 3)

The floral parts arise in acropetal succession (Fig 1) The earliest organ to appear is the glume. It looks like a small papilla when just formed, but as it increases in size becomes slightly dilated at the base. Immediately after its formation the single stamen and carpel arise in quick succession. Young stamen is a club shaped structure (Fig 2) which soon develops into an anther

and a filament (Fig 3) The filament is long and persists even after the shedding of the anther The last organ to appear is the carpel which arises as a complete ring around the torus. It grows quickly to form an elongated style with a constricted base and a slight dilation just above it. As the flower grows old the basal dilated part of the style increases and forms a downwardly projecting ring (Fig 8) The style has a narrow canal. There are two stigmatic lobes differentiated quite early which become elongated and hairy A single ovule develops at the base of the ovarian cavity (Figs. 4-8)

MICROSPOROGENESIS

The young anther consists of a homogenous mass of parenchyma cells. It is slightly reniform in cross section but soon becomes four lobed.

Sections of young anthers show that the archesporium is a single vertical row of 4 to 6 hypodermal cells (Figs. 9 & 10) Each archesporial cell divides periclinally to form a small outer parietal cell and a large inner primary sporogenous cell with dense cytoplasm and a conspicuous nucleus. The primary parietal cell by further periclinal and anticlinal divisions forms a wall of three layers which surround a small group of sporogenous cells (Figs. 11 & 12) The innermost layer of parietal tissue is the glandular tapetum whose cells remain *in situ* till they are finally absorbed. The tapetal cells are uninucleate throughout (Fig 13) The cells of the single middle layer gradually become flattened and ultimately disorganize completely (Fig 13) At the shedding stage the endothecial cells show uniformly thickened inner wall from which finger like prongs of thickenings run along the radial wall to reach the outer wall These prongs are twisted in a left handed manner (Fig 14) The cells of the anther epidermis become flattened and slightly lignified

The primary sporogenous cells divide repeatedly and give rise to spore mother cells which are compact and polygonal in outline (Fig 12) Each spore mother cell undergoes two reduction divisions giving rise to four haploid nuclei (Figs. 15-19) The haploid number of chromosomes is five (Fig 18) of which one is smaller in size (Tanaka, 1939) As there is no quadripartition of the mother cell the quartet nuclei which are of the same size when just formed, lie somewhere in its centre (Fig 19) Of these four nuclei one remains in the centre, increases slightly in size due to increase in nucleoplasm and forms the functional microspore nucleus. The remaining three nuclei move towards the inner side of the mother cell where they gradually degenerate without undergoing any further divisions (Fig 20) A feeble septum is formed between the enlarging nucleus and the three degenerating nuclei which have travelled to a corner in the mother cell. Tanaka (1941) has also reported the same for *Fimbristylis* The three micronuclei become closely pressed against the wall (Fig 21) and by the time the generative cell begins to divide, they degenerate completely (Fig 22)

Male Gametophyte

The young pollen grain has an alveolar cytoplasm. Some of the vacuoles unite to form larger vacuoles. The microspore nucleus becomes displaced towards the periphery where it divides into a large vegetative and a small generative cell. The generative cell just after its formation lies towards the narrow end of the pollen grain (Fig 21) Gradually it migrates and finally becomes situated in the outermost position in the pollen mother cell. Usually the pollen grains are bicelled at the time of shedding but occasionally the generative cell divides before shedding forming two male cells (Fig 22 25)

Ovule

Before the floral organs develop fully a small protuberence appears at the bottom of the ovarian cavity This increases graduall in size and begins to bend away from the mother axis. Soon the rudiments for the integuments appear at its base one after the other These grow rapidly in size and by the time the megaspores are formed and the ovule is anatropous, the integuments reach their maximum development (Figs. 4-8 & 29) The ovule is crassinucellate and bitegmic and both the integuments are two layered. The inner integument alone forms the micropyle (Fig 8 & 29)

Megasporogenesis

There is a single large hypodermal archesporial cell (Fig 26) which by a periclinal division forms a small outer primary parietal cell and a large inner megaspore mother cell (Fig 27) the latter becomes deep seated on account of further anticlinal and periclinal divisions in the primary parietal cell The megaspore mother cell elongates with its nucleus situated in the centre (Fig.28) The nucleus undergoes two reduction divisions each of which is followed by wall formation thus giving rise first to a dyad stage and then a linear tetrad of megaspores (Fig 29) The chalazal megaspore becomes functional and the micropylar megaspores gradually degenerate. The degenerating megaspores persist even after the organization of the mature embryo sac. (Fig 34)

Embryo Sac

The megaspore nucleus divides and the bi tetra and octo-nucleate stages of the embryo sac are formed rap dly (Figs 30-34) The development of the embryo sac conforms to the *Polygonum* type. It is elongated, broad at the micropylar and tapering at the chalazal end. The antipodals are definite cells (Fig 34) The polar nuclei which do not fuse for a considerable time lie somewhere in the centre of the embryo sac

Conclusion

The foregoing account shows that the development of the female gametophyte is of the *Polygonum* type. There are two interesting features noticed in

and a filament (Fig 3) The filament is long and persists even after the ding of the anther The last organ to appear is the carpel which aris complete ring around the torus. It grows quickly to form an elongated with a constricted base and a slight dilation just above it. As the grows old the basal dilated part of the style increases and forms a wardly projecting ring (Fig 8) The style has a narrow canal. Th two stigmatic lobes differentiated quite early which become elongated ar A single ovule develops at the base of the ovarian cavity (Figs. 4-8)

Microsporogenesis

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4 No quadripartition of the microspore mother cell takes place. Only a feeble septum develops between the functional nucleus and the three nonfunctional nuclei but there is none between the latter. The nonfunctional nuclei degenerate before the division of the generative cell. The mature pollen grains at the shedding stage are usually two celled but occasionally they are three celled.

5 The haploid number is five.

6 The ovule is bitegmic and crassinucellate. The inner integument forms the micropyle.

7 The archesporium is single celled and hypodermal. There is a well developed parietal tissue. The megaspore mother cell develops a linear tetrad of megaspores of which the chalazal one is functional.

8 The mature embryo sac is 8 nucleate and its development conforms to the *Polygonum* type.

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LEGEND

Fig 1-8 Organogeny of flower and development of ovule. (An anther Br Bract Cp, Carpel Fl, filament Fp flower primordium Op, ovular primordium St, stamen.) Fig 1 L. S tip of floral axis showing acropetal arrangement of bracts and flower primordia. Fig 2 Same showing initiation of stamen, carpel and ovular primordium. Fig 3 Showing differentiation of stamen into filament and anther and further development of carpellary wall and ovular primordium. Fig 4 Protuberence of ovular primordium at the base of ovarian cavity Figs. 5-7 Differentiation of integuments and bending of ovule. Fig 8. Anatropus ovule with well developed integuments and a mature embryo sac note the dilated base of the style

Fig 9-25 Microsporogenesis and development of male gametophyte. Fig 9 c.s portion of anther lobe showing single hypodermal archesporial cell. Fig 10 *l.s* portion of anther lobe showing single vertical row of five archesporial cells. Fig 11 c.s anther lobe showing porogenous cells surrounded by two wall layers. Fig 12. c.s anther lobe showing spore mother cells surrounded by three wall layers note the differentiated tapetum. Fig 13 s anther lobe showing degenerating tapetum and binucleate pollen grains. Fig 14 c.s a part of anther wall showing fibrous thickening in edothecium and two pollen grains. Fig 15 microspore mother cell. Figs. 16 & 17 stages in the first meiotic division of mother cell. Fig 18. two nucleate mother cell Fig 19 four nucleate mother cell note that all nuclei are lying in centre of mother cell. Fig 20 young pollen grain with functional nucleus in centre and three nonfunctional

nuclei lying in one corner separated from the former by a feeble septum. Fig 21 two celled pollen grain. Fig 22 two celled pollen grain just before division of sickle shaped generative cell. Fig 23 & 24 stages in division of generative cell. Fig 25 a mature pollen grain with two male cells and vegetative cell.

Figs. 26-34 Megasporogenesis and development of female gametophyte. Fig 26 hypodermal archesporium. Fig 27 megaspore mother cell and a parietal cell. Fig 28. elongated megaspore mother cell with its nucleus in centre. Fig 29 *Ls* ovule with a linear tetrad of megaspores. Fig 30 functional megaspore in mitotic division and upper three degenerating magaspores. Fig 31 binucleate embryo sac. Fig 32. tetranucleate embryo sac. Fig 33 third division of embryo sac nuclei. Fig 34 mature embryo sac and persisting degenerating megaspores.

References

1 Dnyansagar, V R. & Tiwari D K. 1956a Microsporogenesis in Fimbristylis quinquangularis Kunth. Curr Sci. 25 16.

2. Dnyansagar, V R. & Tiwari, D.K. 1956b Sporogenesis and Gametophytes of Fimbristylis. quinquangularis Kunth. *Bull Bot Soc Univ Sagar* 8 3-6.

3 Khanna, P 1956 A Contribution to the embryology of Cyperus rotundus *Linn. Proc 43rd Ind Sci. Cong Pt. III* 236-237

4 Padhye, M. D & Moharir, S. K. 1958 Studies in Embryology of Cyperus tegetum Roxb. Proc. Ind. Academy of Sciences, 48 89-95

5 Schnarf K. 1931 ; Vergleichende Embryologie der Angiospermen. Berlin

6. Tanaka, N 1937 Chromosome studies in Cyperaceae. I Cytologia, F jil Jub. Vol. pp. 814-821

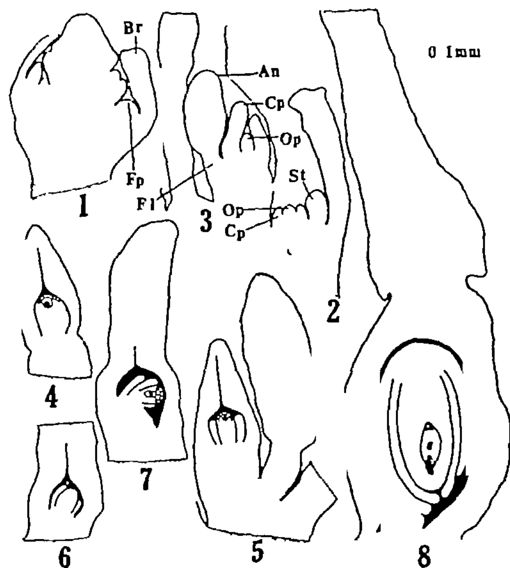
Tanaka, N 1939a ; Chromosome studies in Cyperaceae. IV Chromosome number in *Carex* species. Cytologia 10 51-56.

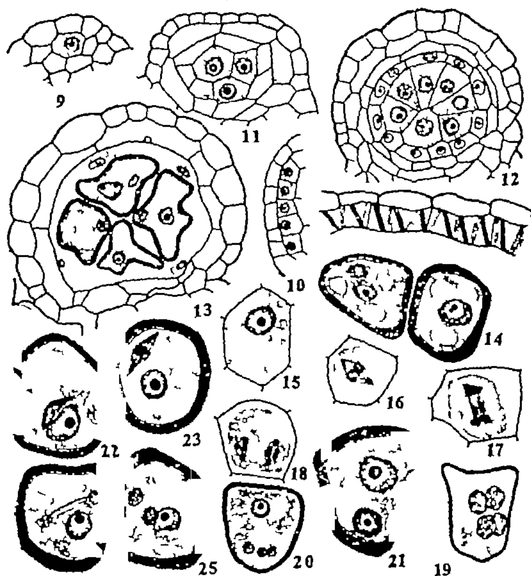
8. Tanaka, N 1939b Chromosome Studies in Cyperaceae. VII Chromosome number and pollen development of *Fimbristylis* Bot Mag Tokyo. 53 480-487

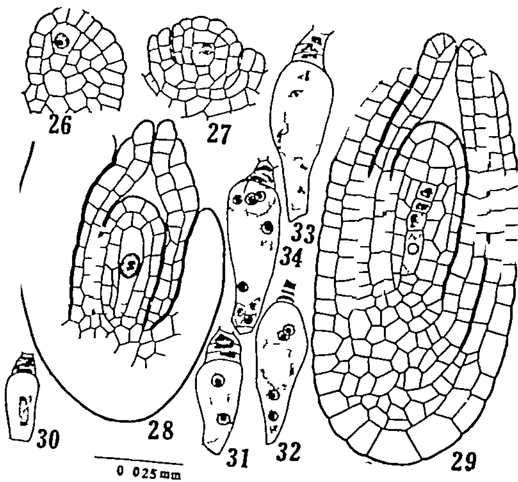
9 Tanaka, N 1940 ; Chromosome studies in Cyperaceae. VI Pollen development and additional evidence for the compound chromosome in *Scirpus lacustris* L. *Ibid.* 10 ; 348-62.

10. Tanaka N 1941 Chromosome studies in Cyperaceae. XII Pollen development in five genera, with special reference to *Rhynchospora*. Bot. Mag Tokyo 55 ; 55-65.

* Quoted from Tanaka 1940


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Suction pressure of the seedlings was estimated by the plasmolytic method by noting changes in fresh weight of the seedlings at 25°C, immersed in graded solutions of cane sugar The observations were recorded in triplicates and means are considered for drawing the conclusions.

Experimental Findings

The data on growth of roots and of plumule are included in Table 1 and 2 respectively

Table 1

Influence of isosmatic concentrations of NaCl and Sucrose on root growth (in mm) in wheat C.591

Age in hours	Control	NaCl	Sucrose	Mean	C. D *
48	3 8	2 8	0 8	2 2	
72	24 0	17 8	18 0	19 9	10 4
96	93 4	56 4	69 4	73 1	
Mean	40 4	25 4	29 4		
C D		10 4			
C D for Interaction		—	—		

*Critical difference at 5 % probability

It is indicated that root-growth was depressed initially to a greater extent by Sucrose than NaCl but the depression was almost equal at 72 hours after sowing On the other hand at 96 hours relatively higher depression was noticed by NaCl.

Table 2

Influence of isosmatic concentration of NaCl and Sucrose on plumule growth (in mm) in wheat C.591

Age in hours	Control	NaCl	Sucrose	Mean	C. D *
72	5 8	4 4	4 0	4 4	
96	13 8	9 2	8 8	10 6	0 25
Mean	9 6	6 8	6 4		
C D		0 31			
C.D for Interaction		0 44			

*Critical difference at 5% probability

A lowering in growth of plumule was evident at 72 hours and again a 96 hours the depression was more or less equal by the two substances.

Observations on suction pressure of the seedlings are given in Table 3.

Table 3

Influence of osmotic concentration of NaCl and Sucrose on Suction Pressure of wheat C.591 seedlings

(Mean of 3 Values)

Age in hours	Suction Pressure in atmospheres		
	Control	NaCl	Sucrose
24	10 1	13 9	15 1
48	3 1	9 5	6 0
72	2 9	4 5	3 3
96	0 9	1 9	1 1

Note Data was recorded at 20 ± 1°C.

The following features are revealed

i. In the seedlings supplied with water (control) a decrease in suction pressure with the age is quite apparent considerable decreases are noticed between 24 and 48 hours and again from 72 to 96 hours suction pressure remained more or less steady between 48 to 72 hours after sowing

(ii) Supplying the seedling with NaCl or Sucrose an increase in suction pressure is discernable, but the values differ for the two substances.

(iii) Lowering in suction pressure of NaCl fed seedlings is more or less gradual with advance in seedling age but a rapid decline was noted for Sucrose particularly between 24 to 48 hours

Discussion

The problem of soil salinity has often been considered synonymous with increasing soil-moisture stress this assumption is based on the absence of specific symptoms associated with Na^+ or Cl^- accumulation in species sensitive to these ions However a deep insight into the problem reveals that the deleterious effect on plant growth may not wholly be osmotic, though the mechanism of chloride toxicity still remains unknown.

In the present study influence of NaCl on early growth of wheat (C. 591) seedlings alongwith presumably non-toxic Sucrose, at isosmotic concentrations has been investigated It is observed that initial reduction in growth of ... followed by a tendency to recover is exhibited by the two substances

higher magnitude of initial reduction coupled with no relapse to inhibition is observed for Sucrose growth of plumule is, however depressed almost to the same level.

Percentage inhibition values on control (distilled water supply) are given below for comparison.

Time in hours	Root growth		Plumule growth	
	NaCl	Sucrose	NaCl	Sucrose
48	47	79		
72	26	25	24	31
96	40	26	33	36

Had the operating factor in growth depression been osmotic, the trend and magnitude of inhibition at isosmotic concentration, irrespective of the solute should be more or less identical The very evidence that repalse to inhibition occurs with chloride alone adds weight to the assumption that replaced inhibition may be due to accumulation of Na^+ and Cl ions to the level toxic to the growth of roots. Toxic effects specific to chloride injury have been reported by a few workers

Uhvits (1946) investigating the influence of NaCl and Mannitol on germination of alfalfa/seeds indicated that decrease in rate of percentage germination as a result of moistening the seeds with either NaCl or Mannitol can be ascribed to osmotic effect of the solution upon the entry of water into the seeds the more severe reduction in the germination percentage brought about by the use of NaCl can be attributed to the injury caused by the accumulation to toxic amounts of chloride within the same Khudairi (1958) studying the effect of NaCl and sugar solutions on germination of date-palm, noted that sugar solutions with osmotic pressure similar to those of NaCl solutions cause much more inhibition to seed germination Bhardwaj (1959) reported upsetting of carbohydrate metabolism during early growth of wheat seedlings which is perhaps due to the toxic effect of the accumulated ions.

In the present study it is interesting to note variations in the suction pressure of seedlings fed on NaCl or Sucrose percentages on control, (distilled water supply) at each period of observation are tabulated below

Treatment	Hours after sowing			
	24	48	72	96
NaCl	138	306	115	211
Sucrose	150	191	114	122

It is evident that the presence of NaCl or Sucrose in the external medium enhances the suction pressure of the seedlings the effect is more pronounced with NaCl Since the suction pressure is the index for the capacity of water uptake it may be conceived that the initial effect tends to be osmotic irrespective of nature of the solute (NaCl or Sucrose) Comparatively higher values for NaCl at 48 hours onwards signifies the increased absorption of chloride ions which on accumulation to toxic level manifest the depressing influence on growth.

SUMMARY

The present investigation relates to the effect of supplying NaCl and Sucrose, in isosmotic concentration of 4 atmospheres, on early growth of wheat (C. 591) seedlings. The seedlings were raised in test-tubes supplied with test solution or distilled water (control) and observations on total length of roots and of plumule were recorded at 24 hourly intervals upto 4 days after sowing. Suction pressure of the seedlings, following the plasmolytic method was also estimated at each period of observation The data were analysed statistically

The results reveal that the growth of wheat seedlings was depressed by supplying isosmotic solutions of NaCl or Sucrose, though the magnitude and nature of depression varied with the substrate used Root-growth was lowered initially to a greater extent by Sucrose but the effect was reversed later on at the end of four days NaCl was relatively more depressing Suction pressure was always higher when the seedlings were grown in Sucrose or NaCl the values for NaCl were comparatively higher

It is concluded from the present study that the effect of supplying NaCl in a harmful concentration tends to be initially osmotic resulting in decreased uptake of water but ions (Na^+ and Cl^-) on accumulation exhibit toxic effects, not comparable to that of sucrose.

ACKNOWLEDGEMENTS

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REFERENCES

1 Bernstein L. and Hayward, H. E. (1958) Physiology of Salt Tolerance An Rev Plt. Physiol. 9 25-46.

2 Bhardwaj, S. N. (1959) Influence of NaCl and Na_2CO_3 on some aspects of Carbohydrate Metabolism in wheat. Ind. Bot. Soc., Memoir 2 73-78

3 Bhardwaj, S N (1961) Physiological studies on salt-tolerance in crop plants. X Effect of NaCl and Na_2CO_3 on early seedling growth of wheat and gram. Nat Acad. (B) 31 143-155.

4 Bhardwaj, S N and Rao I. M. (1958) : Physiological studies on salt-tolerance in wheat and gram. IV Influence of NaCl and Na_2Co_3 on early seedling growth and metabolism in wheat and gram. Proc. Seminar on Recent advances in the study of Plant metabolism, Allahabad Univ p. 105-107

5 Bhardwaj, S. N and Rao, I M. (1960) : Physiological studies on salt-tolerance in crop plants. IX Effect of NaCl and Na_2Co_3 on seedling respiration and growth of wheat and gram varieties. Ind. Jour Plt. Physiol. 3 : 56-71

6 Khudairi, A. K. (1958) : Studies on germination of date-palm seeds. The effect of sodium chloride. Physiol. Planta. 11 : 16-22.

7 Uhvits, R. (1946) Effect of osmotic pressure on water absorption and germination of alfalfa seeds. Amer Jour Bot., 33 : 278-285

THE ENDOSKELETON OF *BAGARIUS BAGARIUS* (HAM.)
Part I—The Skull*

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INTRODUCTION

The fish dates back to the Tertiary period and has existed through the great expanse of time without any appreciable change in its appearance. As such it has been called by Hora (1939) a *Living Fossil*. The fish is interesting as the family to which it belongs forms the parent stock of all catfishes. By Hora (1953) the family is said to have arisen in Europe in Palaeocene from where it spread in Eocene to Africa, South America and Far East. Its incursion into India took place later in the Tertiary period.

It has been considered worthwhile to study the morphology of this interesting living fossil. In the present paper an account on the skull of the fish is being given and the subsequent papers will be devoted to the rest of morphology.

I take this opportunity to thank Dr B. M. Sinha for guiding the work at every step. My acknowledgements are also due to the authorities of the college for providing me necessary facilities for th work.

HISTORICAL RESUME

With respect to complete osteology of fishes references may be made to McMurrich (1884) on *Ameiurus catus*, Day (1941) on *Ophiocephalus striatus* Sarbahi (1932) on *Labeo rohita* Dharmarajan (1936) on *Otolithes ruber* Chapman (1941 1944 a, b) on *Plecoglossus altivelis Anchoa compressa* and *Aplodinotus zebra*, Phillips (1942) on *Sardinops caerulea* and Nawar (1954) on *Clarias lazera*.

On the skull of fishes attention may be drawn to Wright (1885) on *Hypophthalmus* Ridewood (1904) on Clupeoid fishes and Kindred (1919) on *Ameiurus catus*. Bhimachar (1932 33 34) contributed on the cranial osteology of *Ophiocephalus striatus* and a number of Indian Siluroid fishes. Srinivasachar (1955 58) gave an account of the chondrocranium and osteocranium in *Ophiocephalus* and *Heteropneustes*. Moona (1959 a 1959 b) described the skull of *Setipinna phasa* and *Hilsa ilisha* and Sinha (1959) of *Wallago attu*.

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*Part of th thesis submitted for the M. Sc. degree (1960) in the University of Agra

4 Bhardwaj, S N and Rao, I. M. (1958) : Physiological studies on salt-tolerance in wheat and gram. IV Influence of NaCl and Na_2Co_3 on early seedling growth and metabolism in wheat and gram. Proc. Seminar on Recent advances in the study of Plant metabolism, Allahabad Univ p. 105-107

5 Bhardwaj, S N and Rao, I. M. (1960) : Physiological studies on salt-tolerance in crop plants. IX Effect of NaCl and Na_2Co_3 on seedling respiration and growth of wheat and gram varieties. Ind. Jour Plt. Physiol 3 : 56-71

6 Khudairi A. K. (1958) : Studies on germination of date-palm seeds. The effect of sodium chloride. Physiol. Planta. 11 : 16-22.

7 Uhvits, R. (1946) : Effect of osmotic pressure on water absorption and germination of alfalfa seeds. Amer Jour Bot., 33 : 278-285

condyle cannot be distinguished. It articulates with the parasphenoid exoccipital posttemporal and the complex vertebra

The *exoccipital* (II IV 9) is a small irregular bone in the side wall of the occipital region From its dorsal surface arises a laterally compressed *neural plate* which joins the supraoccipital above the neural crest of complex vertebra behind. A *transverse plate* from its outer side articulates with the supraoccipital and epiotic. A perforation on the ventral side of the bone is for the glossopharyngeal nerve and a wider aperture behind for the vagus nerve. The exoccipital joins the supra-occipital, prootic, pterotic epiotic, basioccipital parasphenoid and neural crest of the complex vertebra.

The Auditory Region

The auditory region is ossified by four replacing bones, the prootic epiotic, sphenotic and pterotic. The opisthotic is absent in the fish

The *prootic* (II, IV 7) is a flat squarish bone on the ventral side of the auditory region Along its anterior face, it bears a depression which forms a part of the fenestra for the fifth and seventh cranial nerves It is grooved posteriorly along the cranial surface for th horizontal canal of internal ear The prootic articulates with the pleurosphenoid, parasphenoid, sphenotic pterotic and exoccipital

The *epiotic* (II IV-9) is situated at the posterior end of the auditory region It is roughly triangular n form with th apex produced into a spine A narrow tunnel along its inner surface lodges a part of the horizontal semicircular canal of the ear It articulates with the pterotic supraoccipital posttemporal and exoccipital

The *Sphenotic* (I II III IV 5) is ossified in the anterolateral region of the auditory capsule. It is made up of a thick flat basal part and a forwardly directed process The lower surface of the basal part bears a groove, which in continuity with the groove in the pterotic forms a depression for the head of hyomandibula. The sphenotic articulates with the frontal, pterotic and supraoccipital.

The *pterotic* (I II III IV-4) is located n the posterolateral region of the auditory capsul It consists of the main part and a backwardly directed pterotic process. The main part encloses a part of the horizontal semicircular canal of the internal ear and it joins the sphenotics, supraoccipital prootic, epiotic and exoccipital. The pterotic process is pointed and is applied on the outer side of the posttemporal.

The sphenoidal Region

The sphenoidal region is ossified by the paired frontals and pleurosphenoids and the median orbitosphenoid basisphenoid and

parasphenoid. The parietals are absent in the fish. The basisphenoid, pleurosphenoids and orbitosphenoid are replacing bones, while the frontals and parasphenoid are investing ones.

The *frontal* (I, III IV 11) is a well developed flat bone, which roofs over the sphenoidal region. On the innerside it is separated for the major part of its length with the other frontal by an elongated cranial fontanelle. On its outerside in the posterior region of the bone is a small notch, which forms the inner half of the poorly formed orbit. The frontal articulates with the ethmoid lateral ethmoid supraoccipital and sphenotic.

The *pleurosphenoid* (II IV 14) lies below the posterior part of the frontal It is irregular in form bearing a deep vacuity beneath its posterior half The inner-margin of the vacuity is raised up and joins the similar part from other pleurosphenoid. Running obliquely forward along the posterior edge of the vacuity is a ridge, on which is applied the lateral process of basisphenoid. The bone articulates with the orbitosphenoid frontal sphenotic and pterotic.

The *parasphenoid* (II, IV 10) is an elongated bone distinguished into a squarish body and a forwardly directed long arm. The bone extends in front upto the ethmoidal region and articulates with the lateral ethmoids, vomer and ventral plate of the ethmoid. It continues behind upto the occipital region and interdigitates with the basioccipital. On the outer side it articulates with the prootics and exoccipitals.

The *basisphenoid* (IV 10) is a rhomboidal bone produced into three processes in front Its median process is attached to the upper side of the para sphenoid and each lateral process is applied to the ventral ridge of the pleurosphenoid of its side. The bone is intimately connected to the cranial surface of the parasphenoid.

The *orbitosphenoid* (II IV 13) is formed by the fusion of the paired elements. It constitutes the side wall of the cranium in the anterior part of the sphenoidal region The bone is narrow in the middle and broad at the two ends and is hollow within Its lateral rims run inwards and then outwards to articulate with the inner margins of the frontals. Through the bone runs the olfactory tracts of the brain in their course to the olfactory lobes The orbitosphenoid articulates with the lateral ethmoid, pleurosphenoids, frontals and parasphenoid

The Orbital Region

The orbit is small and is contributed partly by a notch on the side of the frontal. In relation with the orbit are developed the suborbitals and lacrymal, which lie embedded superficially in the muscles

The *suborbitals* (I III IV 5) are four splint like bones extending in a chain below and in front of the eye Through them passes the infraorbital trunk of the lateral line system. The four bones are loosely joined with one another and appear as a single entity

The *lacrymal* (I III IV-6) is a small irregular bone above the anterior head of palatine and the base maxilla. The infraorbital trunk terminates in the bone.

The Ethmoidal Region

The ethmoidal region includes th ethmoid paired lateral ethmoids, nasals and vomer The lateral ethmoids are replacing bones, while the rest are investing ones.

The *ethmoid* (I II III V 1) is a flattened bone on the dorsal side of the ethmoidal region Antero-laterally it is produced into a pair of backwardly directed *anterior horns* The bone gets divided posteriorly into a dorsal and ventral plate separated by a space in which the anterior ends of the olfactory capsules are lodged. From the point where the ethmoid divides into the dorsal and ventral plate, it gives a pair of small *posterior horns* which join the lateral ethmoids. The *dorsal plate* extends back and is produced backwardly into a pair of processes, which enclose the anterior end of the cranial fontanelle The posterior part of the dorsal plate is covered by the anterior ends of the frontals. The *Ventral plate* is also continued back and is separated by a median fissure into two arms. With the two arms is attached the parasphenoid and in the fissure between them lies the stem of the vomer

The *lateral ethmoid* (I II III V 2) lies on the outer side of the ethmoid and frontal. It is like a hammer formed of a shaft and lateral head. The shaft underlies the frontal and the dorsal plate of the ethmoid and it articulates with the parasphenoid ventral plate of ethmoid and orbitosphenoid. On the innerside of the shaft is a vacuity for the posterior end of the olfactory sac The head is very much boney-combed and bears an articular surface for the palatine.

The *vomer* (II V 3) lies on the ventral side of the ethmoidal region. The bone is arrow-shaped consisting of a head and backwardly directed stem. The head is applied to the ventral side of ethmoid, while the laterally flattened stem fits into the fissure in the ventral plate of the ethmoid and in the parasphenoid. The bone is edentulous.

The *nasal* (I III, V 6) is a splint-like small bone on the two sides of the ethmoid and along the inner margin of olfactory capsule The nasal meets in front with the anterior horn of the ethmoid and behind with the frontals. The supraorbital canal of the lateral line system terminates in this bone.

THE VISCERAL SKELETON

The visceral skeleton consists of the mandibular hyoid and five branchial arches. The first four of the branchial arches are complete while the fifth is reduced and without gills

The Mandibular Arch

The mandibular arch which gives rise to upper and lower jaw is ossified by the primary endoskeletal and secondary dermal bones. The palatopterygo-quadrate part is replaced by the palatine metapterygoid and quadrate and invested by the premaxilla maxilla and ectopterygoid. The Meckel's cartilage is ossified by three bones the angular splenial and dentary

The *palatine* (I III V-5) *is a long and piston-like bone, which is aligned* longitudinally on the outer side of the lateral ethmoid. Its head is rounded and bears a facet for articulation with the base of maxilla while the posterior part is laterally compressed and lies free in the musculature Along its inner surface, about the middle of the bone is an articular surface for the lateral ethmoid and over this surface is a process for a second attachment to the lateral ethmoid through a ligament. In association with the maxilla it forms a maxillopalatine mechanism for the abduction and adduction of maxillary barbel and for the ingress and egress of water through the olfactory chamber

The *ectopterygoid* (I,II III V 16) is a flattened plate like bone on the flank of the parasphenoid which is attached to the lateral ethmoid and metapterygoid A process from its front passes below the palatine and lies over the premaxilla and posteriorly it articulates with the hyomandibula. On the upper side of the bone in the anterior region, is a depression for the lateral ethmoid.

The *metapterygoid* (I II III V 15) is a flattened plate like bone on the outerside of ectopterygoid. It articulates in front with the premaxilla and is produced behind into two processes, one articulating with the hyomandibula and the other meeting the quadrate on the outer side of the hyomandibula.

The *quadrate* (I II III V 14) is a stout flat bone with a condyle on its outerside for the articulation of the lower jaw On the inner side it articulates with the metapterygoid and hyomandibula.

The *premaxilla* (II III V 10) is a prominent bone formed of two early separable pieces. At the junction of the two pieces and on the upper surface of the bone is a depression for the heads of the maxilla and palatine The bone meets in front in a symphysis with the other premaxilla and articulates posteriorly with the ectopterygoid and metapterygoid. The bone is toothed the teeth of the outer row being small and of the inner row stout and directed inwards.

The *maxilla* (I II III V 13) is poorly developed and edentulous and it takes no part in the formation of the gape of mouth. The bone is rod like with a bifid head. One knob of the head articulates with the palatine and the other fits in the depression in premaxilla. The rest of the bone forms a basal support for the barbel.

The *dentary* (I III V 9) is a stout curved bone and forms the anterior two-third of the lower jaw. Anteriorly it meets in the mandibular symphysis with the bone of other side and posteriorly it articulates with the angular and splenial. On the upper surface of the bone is a double row of incurved teeth those of the outer row being small and of the inner powerfully developed. On the lower side of the bone is a row of pores for the mandibular canal of the lateral line system.

The *angular* (III V 11) is a stout bone, in the proximal part of the lower jaw. At its proximal end is a facet for articulation with the quadrate and on its outer side is applied the dentary. It bears a groove in front for lodging the splenial.

The *splenial* (V 11) is a small splint-like bone, which lies in a groove at the hind end of the dentary and at the front end of the angular with a small part only visible externally.

The Hyoid Arch

The os-innominatum of hyoid arch consists of the hyomandibula and hyoid cornu.

The *hyomandibula* (I II III V 12) is a prominent flattened bone, on the inner side of which is an elongated head for articulation in the facet on sphenotic. On the posterior face of the bone is a condylar head for the operculum. Beyond the condylar head along the posterior outer edge is a surface for articulation of the preoperculum. The bone articulates with the metapterygoid, ectopterygoid quadrate and preoperculum. The hyomandibula suspends the two jaws and the suspensorium is *methyostylic*.

The *hyoid cornu* (VI) is formed of the epihyal, ceratohyal and hypohyal, the interhyal being absent.

The *epihyal* is a triangular bone, which by its apex articulates with the hyomandibula on the innerside of the interoperculum and by broad end joins the ceratohyal. It carries four branchiostegal rays. The *ceratohyal* is slightly longer than epihyal and interdigitates between the epihyal and hypohyal. It supports the remaining seven branchiostegal rays. The *hypohyal* is a small conical bone, attached by the base to its ceratophyal and by apex to the hypohyal of the other side. The *urohyal* lies on the floor of the buccal cavity below the two hypohyals. It is more or less triangular in form with its apex attached to the hypohyals and the broad end directed backwards.

The gill cover which is supported on the hyoid arch, is formed of three investing bones the operculum, interoperculum and preoperculum, the sub-operculum being absent.

The *operculum* (I II III V 4) is more or less a triangular bone, the dorsal narrow end of which bears a socket for the hyomandibula and to the hind end is attached the branchiostegal membrane On its outer surface radiate a number of markings from the apex. The *interoperculum* (I II III V-8) is a scute-like bone, applied on the posterior border of the preoperculum. By the broad hind end it overlaps the operculum and by the front narrow end articulates with the quadrate. The *preoperculum* (I II III V 7) is an elongated curved bone with the outer end blunt and inner pointed. Although a bone of the opercular series, it is more intimately connected with the quadrate and hyomandibula.

The Branchial Arches

The four *branchial arches* (VI) are of the usual type. The upper part of each branchial arch comprises of the pharyngo-branchial and epibranchial and the lower part is formed of the ceratobranchial and hypobranchial. The *pharyngobranchial* is small bony rod, which lies obliquely in the dorsal wall of the pharynx. At its anterior end it is attached to the prootic and at the posterior end to its epibranchial. The first and second pharyngobranchials fuse insensibly with the epibranchials and the fourth lies in front of the third The *epibranchial* is elongated curved bone which is grooved on its upper surface and provided with *gill rakers* on the lower surface Over the fourth pharyngo-branchial is an oval pad of *superior pharyngeal teeth*.

The *ceratobranchial* is an elongated curved bone two and half times longer than the epibranchial. The bone is grooved on the lower side and provided with a double row of gill rakers on the upper surface It extends from the epibranchial to the hypohyal The *hypobranchials* and *basibranchials* are ossified in the median cartilage which extends from the hypohyals to the fifth pair of ceratobranchials. The hypobranchials are oval pieces ossified on the first and second branchial arches only On the third and fourth arches they are represented by cartilaginous precursors only The *basibranchials* are in th form of two bony flattened rods, which lie one behind the other in the median cartilage The first is situated between the first and second pair of hypobranchials and the second behind it

The *fifth arch* is represented by the ceratobranchials only Each is a curved rod flattened into a plate in the anterior half It is provided with well developed teeth along its sides and its flattened part bears fine *inferior pharyngeal* teeth on the upper surface

SUMMARY

1 The cranium is platybasic and has a single cranial fontanelle The vomer is edentulous and basisphenoid is fused with the upper surface of the

parasphenoid. The parietals opisthotics, symplectics suboperculars, interhyals supratemporals and basihyals are absent. The two orbitosphenoids fuse into one bone.

2. The supraoccipital is produced into the occipital process and the basiocc ptal is fused with the complex vertebra obliterating the condyle and foramen magnum. The prootic, epiotic, pterotic and supraoccipital bear a recess for the pars superiors and the basioccipital and exoccipital for the pars inferiors of the internal ear

3. The orbit is very much reduced and the splint like sub-orbital is formed of four loosely joined pieces. The upper jaw is formed by the pre maxillae alone the reduced maxillae serving as support for the maxillary barbels. The lower jaw is contributed by the dentaries angulares and splenials The suspension is methyostylic.

4 The palatine is well developed and with the maxilla forms the maxillo-palatine mechanism for the abduction and adduction of maxillary barbel and movement of water through the olfactory sac. The pterygoid is absent and the metapterygoid and ectopterygoid are well developed.

5 Each ramus of the hyoid arch has an epihyal, a ceratohyal, a hypohyal and eleven branchiostegal rays The urohyal lies below the hypohyals. The first and second pharyngobranchials are fused with their epibranchials, while the fourth pharyngobranchial lies in front of the third. The hypobranchials and basibranchials on the third fourth and fifth branchial arch are not ossified. The fifth branchial arch is represented by ceratobranchials only Over the fourth epibranchials is a pad of superior pharyngeal teeth and over the most o fifth cerato-branchials are the inferior pharyngeal teeth

REFERENCES

1 BHIMACHAR, B. S. 1932. The cranial osteology of *Ophiocephalus striatus* *Jour Mysore Univ.*, 6 72-86
2 BHIMACHAR B S 1933-34 On the morphology of the skull of certain Indian catfishes. *Jour Mysore Uni* 7 233-267
3 CHAPMAN W M. 1941 The osteology and relationship of the Isospondylous fish *Plecoglossus altivelis* *Jour Morph.* 68 149-165.
4 CHAPMAN, W M 1944 a. The osteology of the pacific deep bodied anchovy *Anchoa compressa*. *Jour Morph.*, 74 311-329
5 CHAPMAN W M 1944 b. On the osteology and relationship of South American fish, *Aphyocharax rubra* *Jour Morph.*, 75 149-165.
6 DAY A. L. 1914 The osseous system of *Ophiocephalus striatus*, *Philip. J Sci.* (D) 9
7 DHARMARAJAN M. 1936. The anatomy of *Otolithus ruber* Part I., The Endoskeleton. *Jour Roy Asiat. Soc. Bengal.*, 2 1 72.
8 DE BEER, G R 1937 The development of the vertebrate skull *Oxford Univ Press* 136-141
9 EATON T H. JR. 1948. Form and function in the head of the channel Catfish *Ictalurus lacustris punctatus*. *Jour Morph.* 83 181 194.

10 GREGORY W. K. 1933. Fish skulls. A study of the evolution of natural mechanisms. *Trans. Amer. Phil. Soc.* 23: 1-481

11 HORA, S. L. 1939. The Game fishes of India, Part IV—*Bagarius bagarius*. *Jour. Bomb. Nat. Hist. Soc.* 40: 583-593.

12 HORA S. L. 1955. Distribution of Indian Fishes of the past and their bearing on the geography of India.
2. The extinct fresh water Teleostean fishes of India. *Every day Science*, 1 (2): 105-113.

13 KINDRED J. E. 1919. The skull of the *Ameiurus catus*. Illinois *Biological Monographs*, 5: 1-117

14 McMURRICH J. B. 1884. The osteology of *Ameiurus catus*. *Proc. Canad. Inst. Sci. N. S.* 2; 270-310.

15 MOONA J. C. 1959 a. Studies on the cranial osteology of Indian Clupeoid fishes. I The skull of *Hilsa ilisha*. *Agra Univ. Jour. Research (Sci)* 8 (1): 53-72.

16 MOONA, J. C. 1959 b. The skull of *Setipinna phasa*. *Science and Culture* 24: 585-587

17 PHILLIPS, J. B. 1942. The osteology of the sardine-*Sardinops caerulea*. *Jour. Morph.*, 70: 463-500.

18 RIDEWOOD W. C. 1904. Cranial osteology of Clupeoid fishes. *Proc. Zool. Soc. London* pp. 448-493.

19 RAMASWAMI D. S. 1955. Skeleton of the cyprinoid fishes in relation to the phylogenetic studies, 7. The skull and Weberian apparatus in the subfamily Cyprininae (Cyprinidae). *Acta Zool. Stockholm.* 36: 199-240.

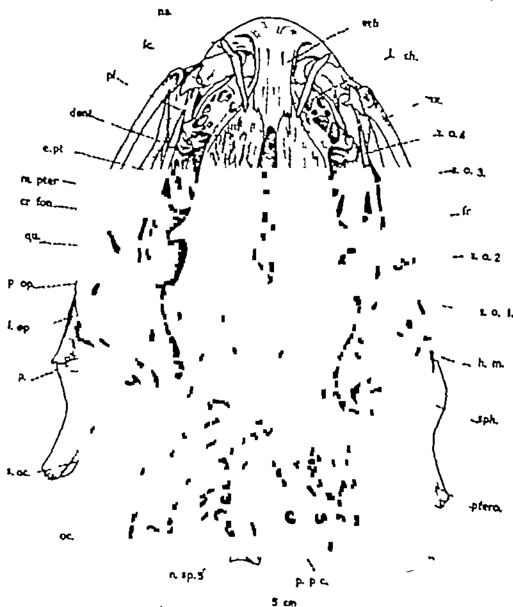
20 SARBAHI, D. S. 1932. The endoskeleton of *Labeo rohita*. *Jour. Roy. Asiat. Soc. Bengal N. S.*, 28: 195-347

21 SRIVASTAVA, B. M. 1959. The endoskeleton of *Wallago attu*. Part I—Skull. *Jour. Asiat. Soc.* 1: 1-14

22 SRINIVASACHAR, H. R. 1955. The skull of *Ophicephalus*. *Proc. Ind. Acad. Sci. India.*, 42: 226-237

23 SRINIVASACHAR H. R. 1958. Development of the skull in catfishes. Part V—Develpment of skull in *Heteropneustes fossilis*. *Proc. Nat. Inst. Sci. India.* 24: 165-190.

24 WRIGHT R. R. 1885. On the skull and auditory region of the Siluroid, *Hypophthalmus*. *Trans. Roy. Soc. Canada.* 107-118.



Dorsal view of the skull

cr fon., cranial fontanelle *dent.*, dentury; *e. ptery.*, ectopterygoid; *eth* ethmoid; *fr* frontal; *h. m.*, hyomandibula; *i. op* interoperculum; *lc.* lacrymal *l. eth.*, lateral ethmoid *mx* maxilla; *m. pter* metapterygoid; *na.* nasal n. sp. 5., neural spine of fifth vertebra; *op.* operculum *oc. pr* occipital process *pl.*, palatine *p. p. c.*, parapophysis of the complex vertebra *p op* preoperculum; *ptero.*, pterotic *pt t.*, posttemporal *qu.*, quadrate *sb orb 1-4* first, second third and fourth sub orbitals; *s. oc.*, supraoccipital *sphe.*, sphenotic;

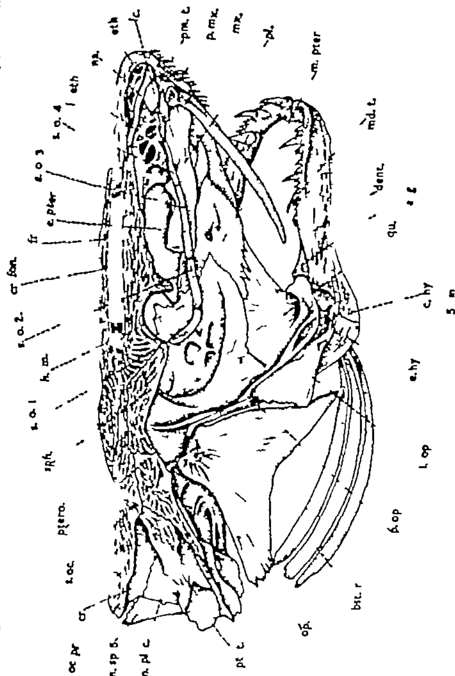
B. bagarius (Ham.)

Plate—II

Ventral view of the skull

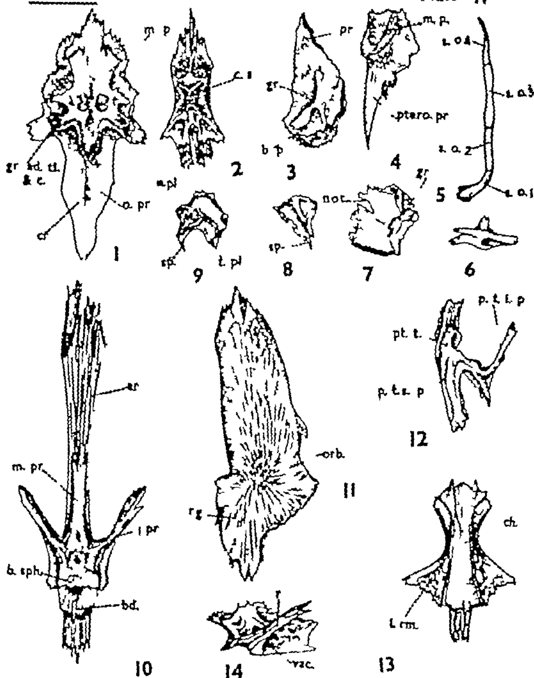
(Lower Jaw removed)

b. oc., basioccipital; *ep. o.* epiotic; *ec. pter.* ectopterygoid; *eth.* ethmoid; *ex. oc.* exoccipital; *h. m.*, hyomandibula; *i. op.*, interoperculum; *l. oc.*, lateral ossification of complex vertebra; *l. eth.*, lateral ethmoid; *mx.* maxilla; *m. pter.* metapterygoid; *o. sph.*, orbito sphenoid; *op.*, operculum; *pm.*, premaxilla; *pm. t.*, premaxillary teeth; *p. op.* preoperculum; *pro.*, prootic; *ptero.*, pterotic; *p. t. i. p.*, post temporal inferior process; *p. t. s. p.* post temporal superior process; *ps.*, parasphenoid; *p. sph.*, pleurosphenoid; *p. p.* parapophysis of complex vertebra; *p. p. 5.* parapophysis of fifth vertebra; *qu.*, quadrate; *sph.* sphenotic; *vo.*, vomer; *vrt. c.*, complex vertebra; *vrt. 5.*, fifth vertebra



Side view of the skull

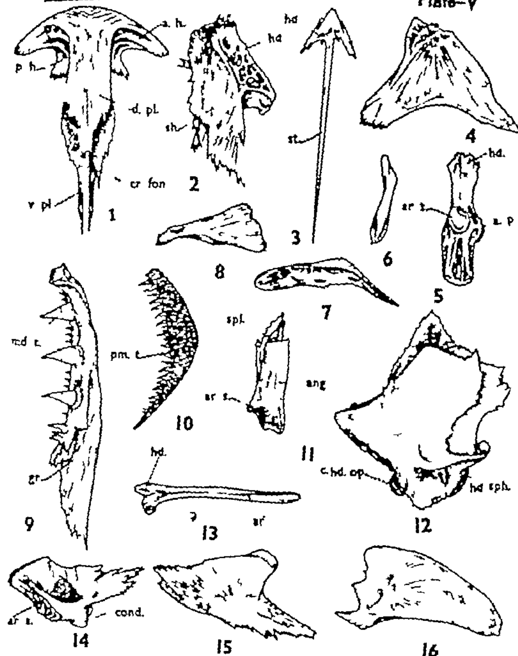
ang angular *brt.* branchiostegal ray *cr* crest, *cr fon.* cranial fontanelle; *c. hy* ceratohyal; *dent* dentary *epter* ectopterygoid *eth.*, ethmoid *e. hy.*, epihyal *f* frontal *h. m.*, hyomandibula *op*, inter operculum; *le* lacrymal; *l eth.*, lateral ethmoid, *mx.*, maxilla; *m. ptery* metapterygoid *md.* mandibular teeth; *na.*, nasal *n. cr* neural crest of complex vertebra; *n. p. 5* neural spine of fifth vertebra *oc. pr* occipital process *op.*, *op* *p op.*, preoperculum; *ptero* pterotic *pt. t* posttemporal *p. mx.*, premaxilla *pm t.*, llary teeth; *pl.*, palatine *qu* quadrate; *oc.* occipital *sph.* sphenotic *s. orb* first second, third and fourth suborbitals.



Bones of the Skull-disarticulated

Fig (1) S pra-occipital Fig (2) Exoccipital Fig (3) Sphenotic Fig (4) Pterotic; Fig (5) Suborbitals Fig. (6) Lacrymal; Fig (7) Prootic Fig (8) Epiotic Fig (9) Exoccipital; Fig (10) Parasphenoid Fig (11) Frontal Fig 12) Posttemporal Fig (13) Orbitosphenoid; Fig (14) Pleu osphenoid

ar arm *b p* basal part *b sph* basisphenoid *bd.* bod *cr* crest *l* cavum sinus imparis *ch* channel *g ad ti & c.*, groove for adipose tissue and semicircular canal; *gr* groove; *l. rm* lateral rim *l pr* lateral process *m p.*, main part *m pr.*, median process; *n. pl.*, neural plate *not* notch *pr* occipital process *orb.*, orbit *p* process *ptero pr* pterotic process; *pt. t* posttemporal *p t p* posttemporal inferior process *p t. p.*, posttemporal superior process; *r.*, ridge of pleurosphenoid *g* ridge of frontal *s. o. 1-4* first, second, third and fourth suborbitals; *sp* spine *t pl* transverse plat ; *vac* vacuity



Bones of the Skull-disarticulated.

Fig. (1) Ethmoid; Fig. (2) Lateral ethmoid Fig. (3) Vomer; Fig (4) Operculum; Fig (5) Palatine, Fig (6) Nasal, Fig (7) Preoperculum; Fig (8) Interoperculum Fig (9) Dentary Fig (10) Premaxilla Fig. (11) Angular; Fig (12) Hyomandibula; Fig (13) Maxilla Fig. (1) Quadrate Fig. (15) Metapterygoid Fig. (16) Ectopterygoid.

ar arm *a. h* anterior horn; *ang* angular; *ar s.*, articular surface; *a. p.*, articular part, *cr fon.*, cranial fontanelle; *cond* condyle *hd op* condylar head for operculum; *d pl* dorsal plate; *gr* , groove *hd.*, head *hd. sph.*, head for sphenotic *md. t* mandibular teeth *p. h.* posterior horn; *pm t.*, premaxillary teeth; *sh.*, shaft, *st.* stem *spl.*, splenial *pl* ventral plate

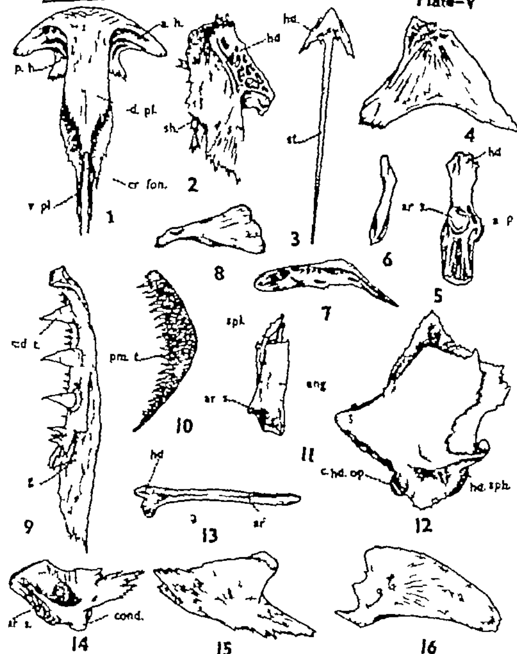
B. bagarius (Ham.)

Plate—IV

Bones of the Skull-disarticulated

Fig. (1) Supra-occipital Fig. (2) Exoccipital Fig. (3) Sphenotic Fig. (4) Pterotic; Fig. (5) Suborbitals Fig. (6) Lacrymal; Fig. (7) Prootic Fig. (8) Epiotic Fig. (9) Exoccipital; Fig. (10) Parasphenoid Fig. (11) Frontal Fig. (12) Posttemporal Fig. (13) Orbitosphenoid; Fig. (14) Pleurosphenoid

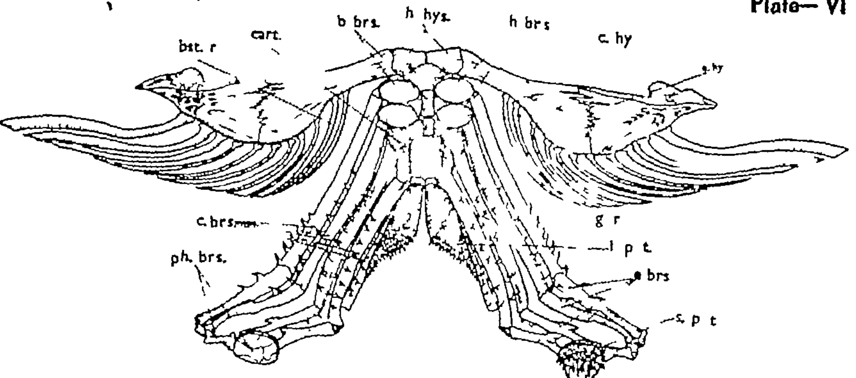
ar. arm *b.p.* basal part *b.sph.* basisphenoid *bd.*, body *cr.* crest *c.s.i.* cavum sinus imparis *ch.* channel *gr. bd. tl. & c.* groove for adipose tissue and semicircular canal; *gr.* groove; *l.rm.* lateral rim *l.pr.* lateral process *m.p.* main part *m.pr.* median process *n.pl.* neural plate *not.* notch *o.pr.* occipital process *orb.*, orbit *pr.* process *ptero.pr.* pterotic process; *pt.t.* posttemporal *p.t.i.p.* posttemporal inferior process *p.t.s.p.*, posttemporal superior process *r.*, ridge of pleurosphenoid *r.g.* ridge of frontal *s.o. 1-4*, first, second, third and fourth suborbitals *sp.* spine *t.pl.* transverse plate *vac.* vacuity



Bones of the Skull-disarticulated.

Fig (1) Ethmoid Fig. (2) Lateral ethmoid; Fig. (3) Vomer Fig (4) Operculum; Fig (5) Palatine; Fig. (6) Nasal, Fig. (7) Preoperculum; Fig. (8) Interoperculum; Fig (9) Dentary Fig. (10) Premaxilla; Fig (11) Angular; Fig (12) Hyomandibula Fig (13) Maxilla; Fig. (14) Quadrate; Fig (15) Metapterygoid Fig (16) Ectopterygoid.

ang angular; ar s., articular surface a. p., articular part; ar arm a. h., anterior horn; cond condyle; c. hd. op condylar head for operculum; d. pl dorsal plate cr fon., cranial fontanelle; g groove; hd. head; hd. sph., head for sphenotic md. t mandibular teeth p. h. posterior horn pm. t premaxillary teeth; sh., shaft st., stem; spl., splenial v. pl ventral plate



The Visceral Skeleton
(Partly opened out)

b brs., basibranchials; *bst* branchiostegal ray; *cart.*, cartilage; *brs.*, ceratobranchials; *c. hy* ceratohyal; *hy.*, epihyal; *e brs.*, epibranchials; *g. r.* gill raker; *h hys* hypohyals; *h brs.*, hypobranchials; *l p t.*, inferior pharyngeal teeth; *ph. brs* pharyngobranchials; *s. p. t* superior pharyngeal teeth.

A NOTE ON THE EFFECT OF MALEIC HYDRAZIDE ON GROWTH AND FLOWERING OF DIANTHUS (*Dianthus Caryophyllus*)

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Maleic hydrazide (M H) is considered to produce wide variety of respon ses depending upon the species, age of the plant and concentration of the chemical employed (Schoene and Hoffman 1949) White (1950) reported temporary inhibiting effect and delayed blooming in certain flowering plants. Naylor and Davis (1950) Moore (1950) Struckmeyer and Beck (1952) Beach and Leopold (1953) Beck *et al* (1957) and Jauhari and Jit (1960) found temporary suspension of stem elongation from terminal buds in some flowering plants. Lateral buds expanded sometime after the terminal bud had been affected.

The desirability of MH in breaking the apical dominance of flowering plants may help in dispensing with the tedious and time consuming practice of manual pinch ng and subsequently encourage a larger number of axillary buds along with a larger number of better flowers. The present investigation was, therefore, carried out to study the response of MH on growth and flower ing of dianthus (*Dianthus caryophyllus*)

MATERIAL AND METHODS

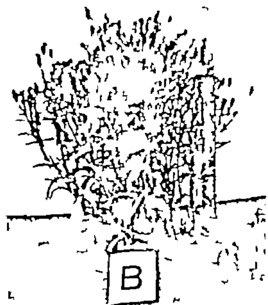
Four week-old seedlings of dianthus were transplanted (1 × 1½') in random selected beds. The design of layout was a simple randomised one with five treatments replicated four times Four concentrations of MH (500 1000, 1500 and 2000 parts per million) were prepared in distilled water (pH 6 0) When the seedl'ngs were three months old, the solutions were sprayed with a hand automiser on the leaves and shoots. Weekly observations regarding different characters of growth and flowering were recorded and continued till the plants completed flowering

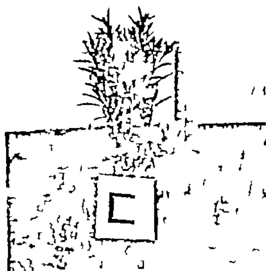
EXPERIMENTAL FINDING

Different treatments brought about marked changes in the vegetative growth and flower production of dianthus (Table 1) The data in the table are the average of five plants under each treatment.

The average height of th differently treated plants was lower than controls (F g A) and the height decreased with increases in concentration. Maximum number of laterals were produced in plants treated with 500 p p. m. (Fig B) In plants subjected to higher concentrati n, there was proportionate reduction in n mber of laterals (Fig C), as compared with the control.


A


B


C


A


B


C

Table 4

Water stable aggregates at the end of the experiment.
Mechanical analysis clay (A)—17 15% silt (B)—8 5%

	Treatments	Clay aggregation			Silt aggregation		
		Dispersed Clay (C)	Aggregated Clay (D)	% Aggregation (E)	Dispersed Clay (F)	Aggregated Clay (G)	% Aggregation (H)
		At 0 1 ppm. Level					
1	Soil alone at start	5 50	11 65	67 93	18 65	1 30	6 516
2	Soil+Organic Matter	4 85	12 30	71 72	18 90	1 70	8 427
3	Soil+Organic Matter+Mo	4 15	13 00	75 86	18 65	2 65	12 441
4	Soil+Organic Matter+B	4 75	12 4[illegible]	72 42	18 20	2 50	12 077
5	Soil+Organic Matter+Zn	5 10	12 05	70 27	18 15	2 20	10 810
6	Soil+Organic Matter+Cu	4 25	12 90	75 21	18 25	2 95	13 915
7	Soil+Organic Matter+Co	5 15	12 00	70 00	17 55	2 40	12 030
		At 0 2 ppm. Level					
8	Soil+Organic Matter+Mo	3 75	13 40	78 13	16 75	4 95	22 811
9	Soil+Organic Matter+B	4 50	12 60	73 41	16 55	4 15	20 000
10	Soil+Organic Matter+Zn	4 70	12 45	72 59	18 15	2 60	12 530
11	Soil+Organic Matter+Cu	4 40	13 15	6 67	18 45	3 00	14 000
12	Soil+Organic Matter+Co	4 75	12 40	72 30	15 75	4 95	23 913

depends upon the decomposition of the organic residue and the change of nutrients in the available forms As decomposition of organic matter is a *microbial process and trace elements play an important part in the activity of* microorganisms so the micronutrients play a very important role in the decomposition of organic matter

As a result of mineralization of organic phosphorus and nitrogen, there had been corresponding increase in the values of available P_2O_5 and NO_3-N during this period As decomposition of organic matter releases locked up energy and make it available for nitrogen fixing organisms, a corresponding increase in the values of total nitrogen was also observed during the period of active decomposition of organic matter Trace elements having some effect or the other on the physiology of the organism affect the decomposition of organic matter The requirement of different trace elements are closely related to the amount of cell material produced thereby increasing the growth and physiological function of organisms. Effect of Mo was more pronounced than the others Mo treated soils showed faster rate of decomposition gain in total nitrogen and faster mineralization of organic nitrogen and phosphorus. This corroborates with the finding of Bortels (1930) Steinberg (1936) Herzinger (1940) Burk and coworkers (1942) and Mulder (1948)

C/N ratio of the treated soil became narrower than that of the original soil. This supports the finding of Waksman (1952) who observed that green manuring lowers the organic matter of the soil and the humus left from the decomposition of green manures does not completely replace the humus lost from the soil as a result of cultivation

Nitrate-nitrogen content of the soil closely corresponds with the decomposition of organic carbon and gain in soil nitrogen Greater nitrification was observed with higher concentration of trace elements. The pronounced effect of Mo and B on nitrification closely corroborates the findings of Bortels (1930) and Steinberg (1941)

There was an increase in the values of available phosphorus of the treated samples over control. In General maximum increase in the values of phosphorus was at 51st day after which they showed fluctuations which may be attributed to the biological and chemical conversion of available phosphorus into nonavailable form. A similar result was observed by Demolon and Parbin (1930) Swenson Cole and Seiling (1949) and Struther and Seiling (1950)

Aggregation of the finer particles gives a fair indication of increased humification in soil treated with trace elements The results thus achieved are in quite agreement with those observed by Joachim and Kandiah (1941) Shrikhande and Pathak (1951) and Pathak and Lehri (1959) Increased aggregation *of silt and clay was observed for the Mo treated soils which further increased with the increase in the concentration.* The higher aggregation in soils treated with Mo at both the concentrations are in agreement with the values of organic carbon total nitrogen C/N ratio nitrate nitrogen and available phosphorus

Summary

Normal cultivated soil from students Instructional Farm Govt. Agricultural College Kanpur was treated with sannai at the rate of 150 mds. per acre. Effect of some trace elements like Mo B Zn Cu and Co at the rate of 0 1 ppm and 0 2 ppm on the decomposition of organic matter was tested It was observed that the decomposition was faster in trace elements treated soil they also fixed more nitrogen than the untreated one. Of these Mo gave best result. C/N ratio of the soil narrowed down with the period of decomposition the rate being faster in the trace elements treated soil. There was an increase in the values of available phosphorus and nitrate nitrogen in soils treated with the trace elements. Increase in the aggregation of the finer particles of the soil was observed at the end of the experimental period in all the treatments Mo at both the concentrations showed highest aggregation

References

1 Ark, P A. (1937) *Proc Amer Soc Hort Sci.* 34 216-2.1
2 Bal, D. V (1925) *J Agri. Sci.* 5.
3 Basu, J K. and Sirur S S. (1943) *Ind J Agri. Sci* 113 66.
4 Bertrand, G. & J villier M. (1911) *C. R Acad Sci. Paris* 152 900-903
5 Bortels, H. (1927); *Biochem. Z* 182 301 358.
6 Bortels, H (1930) *Arch. Mikrobiol* 1 333-342
7 Burk, D. & Coworkers (1942); *J Agri. Res* 65 173-193
8 Demolon A. & Barbier, G (1930) *Ann. Sci Agron. Franc et Etrang* 47 329-363
9 Gollmick, F (1936) *Z. Bakt.* 1193 41 -412.
10 Harper H. J (1924); *Ind Eng Chem* 16 180.
11 Harvey H. W (1939) *J Mar Biol As U K* 23 499-520.
1 H ralinger F (1910) *Pflanzenernahr* 16 141 168
13 Hopkins, E. F (1930) *Soil Sci.* 72 609-610
14 Hopkins, E F (1934) *Mem Cornell. Agri E p Sta.* Ya. 151.
15 Joachim, A. W R. and Kandiah, S. (1941) *Tropical Agriculturist* 95 336
16 Mulder E G (1948) *Plant & Soil* 1 94-179.
17 Pathak, A. N. & Lehri, L. K. (1959) *Ind. Soc Soil Sci* 7 87 90
18 Piper C. S (1940) *J Au Agri Sci.* 6 162 164
19 Raulin, J (1869); *Ann. Sc Nat Bot.* 5 93-299
20 Roberg, M. (1928) *Zbl. Bakt. II* 74 333-371
21 Roberg, M (1932) *Jb Wiss. Bot.* 76 311 332
22 Shrikhande J G and Pathak, A. N (1951) *Ind Soc Soil Sci* 21 401
23 Steinberg R. A (1919) *Amer J Bot* 6 330-372
24 Steinberg, R. A. (1933) *Bull. Torry Bot Cl.* 62 81-90
25 Steinberg, R. A. (1936) *J Agr Res.* 52 439-448
26 Steinberg R. A. (1937) *J Agri. Res* 55 891-902
27 Steinberg, R A. (1941); *J Agri. Res* 62 423-430.
28 Struther P H. and Selling, D N (1950) *Soil Sci.* 69 205-213
29 Swenson, R. M, Cole C V & Sieling D N (1949); *Soil Sci.* 6 3-22.
30 Troug R. (1930) *J Amer Soc. Agron* 22 274
31 Waksman S. A. (1932); *Soil Microbiology Cap* 14 318 320.
32 Waksman S. A., Jhonstone D B. and Carey C. L (1913); *J Marine Res* 2 136-15.
33 Wilkey A. & Black, I A. (1934) *Soil Sci* 37 29.
34 Wolff L. K. and Emmeric, A. (1930); *Biochem. Z* 288 411-458.

STUDIES ON SALMONELLAE FROM DOMESTIC ANIMALS WITH SPECIAL REFERENCE TO POULTRY*

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An investigation was undertaken to isolate and classify the salmonella organisms from domestic animals. In total 1322 fowls, 206 dead-in-shell embryos, 68 pigeons, 110 ducks 455 cattle 290 buffaloes 163 sheep 91 goats, 247 pigs and 18 rats were examined and 24 fowls 7 dead in-shell embryos 1 duck, 8 cattle, 9 buffaloes, 4 goats 3 pigs and 1 rat were found harbour salmonella organisms usually in symptomless carrier state.

Usually four enrichment for example Muller's tetrathionate Kauffmann s modified tetrathionate selenite and hydroquinone broths and three selective media Desoxycholate citrate agar Brilliant green agar and Salmonella shigella agar were used for the isolation of Salmonellae. Kauffmann s modified tetrathionate broth appeared to give slightly better results than others. Desoxycholate citrate agar and Salmonella—Shigella agar were considered to be slightly better than Brilliant green agar Addition of sucrose and/or sallcin to these selective media has been found to give good results. The use of more than one enrichment and several selective media per specimen for isolation of Salmonellae was found to be more satisfactory

In testing suspicious colonies from isolation plates preliminary biochemical tests, polyvalent O sera (A E) and salmonella-genus-specific 0-1 bacteriophage were used and it has been concluded that these all methods in combination with each other or combination of biochemical test with genus-specific 0-1 phage lysis tests may be used with success for screening of salmonella colonies.

In the present investigation 33 strains were isolated with the help of 0-1 phage and all of them proved to be Salmonella It seemed to be a rapid, simple and inexpensive method for the identification of the genus Salmonella. Twenty four strains of Salmonella isolated on the basis of preliminary biochemical reactions were put to 0-1 phage lysis tests and five of them were found to be somewhat resistant to this phage.

Three lysogenic phages were recovered from the isolated salmonella strains. With the help of these three phages fou strains of *Salmonella anatum* of different animal sources were phage typed and divided into three phage types.

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*This is an abstract of the thesis submitted in partial fulfilment of the requirements for the degree of M V Sc (V. L. Sc.) in Bacteriology of the Agra University (April, 1960)

C. D R. I phage from human colitis patient showed lysis only over 5 strains of animal origin.

It appears that the phage pattern of animal origin might be different from that of human origin and the presence of common phage types may indicate cross infections

Attempts to relate fermentation reaction in maltose of strains of the same serological group with the phage types, were made. It was observed that strains belonging to the same serological and fermentation type fermenting maltose within 24 hours, belonged to similar and uniform phage types and they varied if the strains belonged to the other fermentation type in which maltose was fermented late.

The difference in lytic reaction of the phages between strains isolated from normal and diseased conditions were observed. Out of 67 strains of *E. coli* isolated from diseased condition in man and animals, 80% of the strains were lysed by only one or two of the total 64 phages used in phage typing and 20% did not show any lysis. Fifteen strains of *E. coli* O55 B5 group from cases of infantile diarrhoea could not be typed locally out of which according to Nicolle 9 belonged to St Christopher phage type and the rest 6 perhaps belong to some other new phage types the studies of which are still being continued.

Serological studies revealed the presence of 'o groups 2,8 9 1o 18 21 23 and 25 in the strains isolated from Madhurikund farm

Pathogenicity trials showed that 36% of the 30 normal strains were pathogenic to mice and 90 % of the 10 strains from inflammed appendices from man showed pathogenicity towards mice indicating that appendicitis in man may be associated with *E. coli* infection.

COLLOID-ELECTROLYTE BOUNDARY AND KOHLRAUSCH'S RELATION $T_A/C_A = T_B/C_B$ FOR IONIC BOUNDARIES

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Abani K. Bhattacharya
Department of Chemistry Agra College Agra

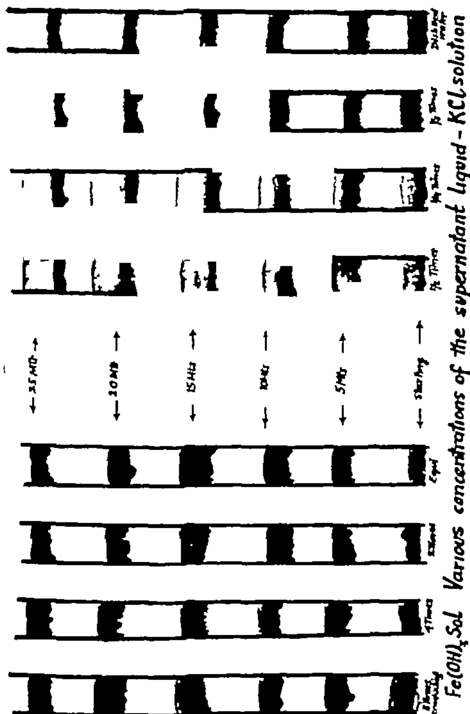
From theoretical considerations Kohlrausch deduced a relation between the transference numbers and the concentrations of the ions which was necessary for the sharpness of the moving boundary formed between the electrolytes AR and BR. It was proved mathematically that the condition which must be made to prevail in the study of boundary movement was given by $T_A/C_A = T_B/C_B$ where T_A and T_B are the transference numbers of the ions A and B and C_A and C_B their concentrations respectively The restoring effect under such conditions plays a very prominent role by which the indicator ion constituent at the boundary moves at the same rate as the leading ion to maintain the desired sharpness It should be noted however that the researches on the moving boundaries have been mainly confined to the mixtures of only two electrolytes with a common ion but the moving boundaries in the systems containing a larger number of constituents have hardly been studied.

By gradually increasing the strength of KCl solution upto the concentration which is equiconducting with the sol it was observed (vide coloured plate) that the faint yellow layer of the boundary was gradually replaced by deep yellow then orange and finally darker read But when the supernatant electrolyte of higher conductivity than that of the sol (4 times to 8 time of the conductivity of the sol.) was used a still darker brown layer was formed below the yellow layer of the sol on passing the current. Gradually the yellow layer was overtaken by the brown layer forming a sharp boundary but the time taken for merging of the yellow layer with the red was longer than when the equiconducting supernatant liquid was used. These observations were explained tentatively in our communication [Koll Zeit. 159 Bd Heft Seite, 7–11 (1958)] but further explanations are necessary to explain such interesting results of the boundary movement. The yellow colour in the boundary may be either due to more dispersed particles of Fe (OH) or it may be due to very low concentration of the soil particles. Kohlrausch (1897) deduced from theoretical considerations a relation between the transference numbers and ionic concentration fo the sharpness of the moving boundary between two electrolytes, AR and BR. This relation is a very important one and has been supported by the boundary movement between electrolytes having a common ion by M Innes, Dennison and Steel and others. The relation is $T_A/C_A = T\ /C_B$ where T_A and T_B are the transference

numbers of the leading and the indicator ion C_A and C_B are their concentrations respectively In the distilled water the concentration of the leading H ion is very small i.e., C_A is very small. Under such conditions the sharpness of the boundary will depend upon the adjustment of the ratio C_A and C_B with transference numbers of the H ion and that of the $Fe(OH)_3$ micelle. If this assumption is justifiable to the electrolyte-colloid boundary then the advancement of the yellow layer only (very dilute layer) and negligible movement of the more concentrated layer of $Fe(OH)_3$ may be explained because C_A is a very small quantity T_A/C_A will be very large hence T_B/C_B to attain this value C_B should be very small which means that the concentration of the indicator layer will remain low throughout the movement according to Kohlrausch. This has actually been observed and seems to be the probable reason why the more concentrated reddish brown layer of Fe $(OH)_3$ sol does not seem to move at all under such conditions where distilled water is taken as supernatant liquid. The presence of hydroxyl ion has been assumed to have no positive role to play because of its extremely small concentration

According to this theory of Kohlrausch, as the value of the ratio T_A/C_A will decrease, as C_A the concentration of the supernatant layer of KCl, is increased the ratio T_B/C_B should also decrease This means that the concentration C_B of the advancing sol column will be greater than in the case when distilled water was used as supernatant liquid. Thus it is evident that more particles from the sol column will advance to follow the leading ion, making the boundary of greater intensity and sharpness, the best result being attained when the supernatant layer is equiconducting as had been suggested by previous authors in this field

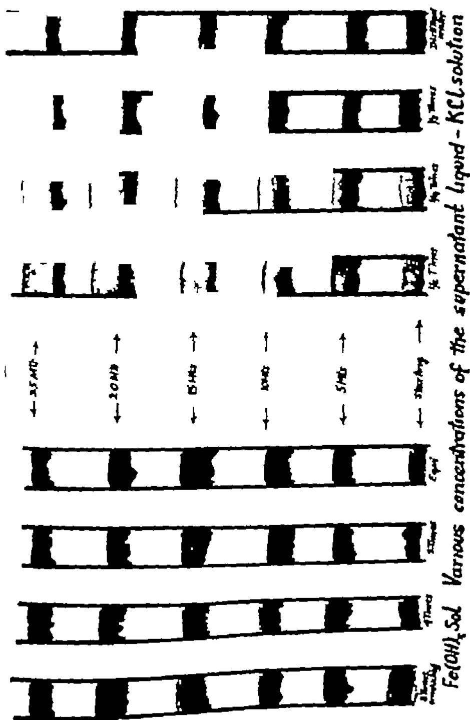
Although the movement of the yellow layer and reddish brown layer has been explained satisfactorily by the Kohlrausch's relation $T_A/C_A = T_B/C_B$ it still remains an interesting field of investigation to find out whether the gradual changes of the colour of the advancing column from (faint yellow→light yellow→yellow→orange→red and finally to dark red) under the aforesaid conditions could also be due to the haterogeneous composition of the system. The possibility of relatively small or big size particles of different shape and characteristics cannot be altogether ignored with the result that slow and fast particles may simultaneously participate in the electrophoretic phenomenon.


Fe(OH)$_3$ Sol
Various concentrations of the supernatant liquid - KCl solution
35 Hrs
30 Hrs
15 Hrs
10 Hrs
5 Hrs
Starting

numbers of the leading and the indicator ion C_A and C_B are their concentrations respectively In the distilled water the concentration of the leading H ion is very small i.e. C_A is very small. Under such conditions the sharpness of the boundary will depend upon the adjustment of the ratio C_A and C_B with transference numbers, of the H ion and that of the $Fe(OH)_3$ micelle. If this assumption is justifiable to the electrolyte-colloid boundary then the advancement of the yellow layer only (very dilute layer) and negligible movement of the more concentrated layer of $Fe(OH)_3$ may be explained because C_A is a very small quantity T_A/C_A will be very large hence T_B/C_B to attain this value C_B should be very small which means that the concentration of the indicator layer will remain low throughout the movement according to Kohlrausch. This has actually been observed and seems to be the probable reason why the more concentrated reddish brown layer of $Fe(OH)_3$ sol does not seem to move at all under such conditions where distilled water is taken as supernatant liquid The presence of hydroxyl ion has been assumed to have no positive role to play because of its extremely small concentration

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Fe(OH)$_3$ Sol
Various concentrations of the supernatant liquid - KCl solution

A NOTE ON THE WING VENATION OF *SPHYRACEPHALA HEARSEYANA* WESTW. (DIOPSIDAE: DIPTERA)

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Hyaline with blackish stigma. Costa (C) extends along the anterior margin beyond the apex to terminate at level with Media (M). Basally Costa (C) bears nine prominent setae, the fourth, seventh, eighth and ninth pointing anterior while the rest posterior. Besides these prominent setae, the Costa is beset with small bristles all along its outer margin. Subcosta (Sc) and the radius (R) have a common base which divides before the humeral cross-vein (h) into two, the anterior subcosta (Sc) and the posterior radius (R). Subcosta (Sc) runs upto one fourth the length of the wing before taking an upward curve to meet the costa. It bears two minute anteriorly directed setae proximal to the humeral cross vein (h). Basally, the radius (R) bears five prominent posteriorly pointed setae and runs as a single vein for a short distance before bifurcating into an anterior radius R_1) and posterior radial sector (Rs). The R_1 runs just below the subcosta (Sc) and meets the costa a little before the middle of the wing margin, enclosing a small, dusted stigma (Stg). The radial sector (Rs) divides into two branches a little before the termination of R_1, the anterior representing R_{2+3} while the posterior constitutes the R_{4+5}. The former runs almost parallel to the anterior wing margin and joins the costa subapically. The R_{4+5} runs straight and meets the costa apically.

Media (M), the cubitus (Cu) and the anal (A) have a common stalk which at level with the alular incision divides into two, the anterior media+cubitus (M+Cu) and the posterior the anal (A). The M+Cu soon divides into an anterior (M) which runs forward and meets the costa at its termination while the posterior (Cu) gives rise to Cu_1 and Cu_2. The Cu_1 stops short of the posterior wing margin but the Cu_2 follows a backward course and meets the anal (A) to end as Cu_2+A in the middle of the third posterior cell (3d PC).

In addition to longitudinal veins there are three distinct cross veins viz.—(i) the humeral cross-vein (h) between costa and the subcosta (ii) the radio-medial (r-m) between radius $_{4+5}$ and the media (M) (iii) the medio-cubitus (m-cu) between the media and the cubitus$_1$ (Cu_1). The wing surface is divided into five closed cells and eight open cells. The closed cells include (i) basal costal cell (BCC) (ii) distal costal cell (DCC) (iii) basal cell (BC) (iv) discal cell (DC) and the (v) cubito-anal cell (Cu-A) while

Contribution No. 93 from the School of Entomology, St. John's College, Agra.

the open cells are (i) subcostal cell (ScC) (ii) Stigma (Stg) (iii) marginal cell (MC) (iv) submarginal cell (SMC) (v) first posterior cell (1st PC) (vi) second posterior cell (2d PC) (vii) third posterior cell (3d PC) and (viii) the axillary cell (AXC) At the base of the wing is a distinct alula (AL)

Wing articulation —The wing articulates with the mesonotum by the help of three axillary sclerites. Besides these is also present a small sclerotised humeral plate at the base of the costa and flat small pubescent lobe, the tegula at the base of the wing The tegula (tg) bears five prominent setae The first axillary (1 AX) somewhat irregular in form with three distinct processes is attached proximally to the anterior notal wing process (ANP) Its anterior bluntly pointed process extends upto the base of the humeral plate the median one stops short at the base of subcosta+radius (Sc+R) while the posterior encroaches upon the area of the median plates. The anterior process gives rise to a prominent ridge running ventrally upto the base of the axillary (1 AX) The second axillary (2 AX) is a quadrangular sclerite, with a distal margin curved over the proximal margin at its posterior end. The 3rd axillary (3 AX) is a large piece and bears a prominent obtusely pointed dorsal process. Proximally it is attached to the posterior notal wing process (PNP) and distally supports the common stalk of M+Cu+A veins. The posterior edge of the axillary membrane forms a thickened fold the axillary cord (AXCO) continuous with the posterior margin of the tergum.

Halteres —The halteres are distinctly divided into three regions a small basal scabellum (SBM) an elongated pedicel (PED) and a flattened rounded terminal lobe, the capitellum (CTM) The scabellum is hinged to metanotum by a ball and socket joint and bears seven rows of sensoria. The pedicel is nearly three times as long as the scabellum, broader basally and narrowing distally It bears a semicircular row of sensoria at its base with three distinct setae on the outer margin. The capitellum is pubescent with a few prominent setae and is almost as long as wide.

SUMMARY

The wing shows the costa, subcosta radius$_1$ radius$_{2+3}$ radius$_{4+5}$ media, cubitus$_1$ cubitus$_2$ and the anal veins lengthwise while the humeral, the radio-medial and the medio-cubital veins crosswise. The costal, subcostal, stigma, marginal, sub-marginal, the three posterior basal, discal and the cubito-anal cells are distinctly marked. The axillary region of the wing is supported by three axillaries a humeral plate and the tegula. The haltere represents three parts, the scabellum, the pedicel and the capitellum

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References

1. Chen, S. H. 1949. Records of Chinese Diopsidae and Celyphidae (Diptera). *Sinensia, Shanghai* 20: 7-18.
2. Comstock, J. H. and Needham, J. G. 1898-99. The Wings of insects. *Amer. Nat.* 32 and 33.
3. Curran, C. H. 1934. Notes and descriptions of African Diptera. *Amer. Mus. Novit. New York.* No. 710.
4. Lameere, A. 1922. Sur la nervation alaire des Insectes. *Bull. Acad. Roy. Belg.* pp. 138-149.
5. Ouchi, Y. *1931.* On two new species of Chinese stalked-eyed flies (Diptera sinica Diopsidae I). *J. Shanghai Sci. Inst.* N.S. 2: 45-48.
6. Seguy, E. 1949. Diopsides de Madagascar. *Mem. Inst. Sc. Madagascar. Tananarive,* 3 A: 165-170.
7. Seguy, E. 1955. Dipteres Diopsides africains nouv aux ou peu connus. *Bull. Inst. Franc. Afrique Dakar.* V 17: 1102-1124.

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ON THE BIOLOGY AND IMMATURE STAGES OF A SAP-SUCKER ON *ZIZIPHUS JUJUBA*, *MONOSTEIRA MINUTULA* MONTANDON, A SPECIES NEW TO INDIA (HEMIPTERA: TINGIDAE)

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CONTENTS

INTRODUCTION

Earlier contributions on the bionomics and life-histories of some Tingidae from India include those of Khan (1945) and Roonwal (1952) on *Teleonemia scrupulosa* Stål, an imported *Lantana* bug; Sharga (1953) on *Monanthia globulifera* Walk., the *Ocimum* lace bug; Mathur (1955) on *Tingis beesoni* Drake, the teak lace bug; Patel and Kulkarney (1955) on *Urentius echinus* Dist., the brinjal lace bug and Livingstone (1959) on *Urentius euonymus* Dist., the hollyhock lace bug. The bionomics, periodicity and the nature of the damage caused to the host plants of various species of Tingids, which periodically attack plants of economic value, have not been fully investigated. An attempt is made here to deal with certain aspects of the bionomics and immature stages of *Monosteira minutula* Montandon. Most of the observations were made in the field.

INCIDENCE

The genus *Monosteira*, with five recorded species, is confined to the Palaearctic Region (Drake 1960), except the species *M. minutula* Montandon which is known from the Ethiopian Region. It is being recorded here for the first time from the Indian faunal limits. In Agra this species has a very limited distribution.

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M. mustela makes its first appearance on *Zizyphus jujuba* (Ber) in the middle of March, when stray adults feed on both sides of the leaves. Multiplication takes place very rapidly so that by the middle of April the whole tree is infested during which period an average of four adults and seven immature insects could be found on a single leaf. Both adults and immature stages attack leaves and buds, the latter get charred while the former become dechlorophylled and wither. During the middle of June when the leaves are shed, no insect could be found, but soon after the monsoon (Middle of July) with the emergence of new foliage the insects also reappear and multiply rapidly. The population again shoots up to the peak during the middle of August but the damage caused to the tree at this time is insignificant as the growth of the fresh shoots is fast. There is a steady decrease in the population during the end of September and the insects altogether disappear by the end of October. *M. mustela* represents a summer species available in Agra and is specific to *Zizyphus jujuba*. The only other species of tingid so far recorded on *Zizyphus* by Menon and Hakk (1959) is *Urentius ziziphifolius*.

DESCRIPTION OF THE INSECT (FIGS. 1-6)

Length 1.5 mm, width 0.8 mm. The following details will supplement the descriptions given by Montandon (1897) —

The buccular lobes (Fig. 1 BU) visible from above on either side of the anteclypeus. Head with five white, blunt spines (tubercles?) one on either side of the ante-clypeus (Fig. 1 AC) on the clypeal groove, two spines on the vertex (Fig. 1 V) one on either side just below the level of the eye and the median spine at the base of the anteclypeus. Eyes carmine red in newly emerged adults and later turn to brown. Antennae four segmented, the scape and pedicel of equal length and bare, the third segment more than half as long as the entire antenna, the terminal segment brownish, punctate and as long as the first two combined, antennal tubercles blunt and incurved ventrally, the scape and the antennal tubercle being shielded by the anterior outer expansion of the buccula (Fig. 5 AOB). Posterior half of the head covered by the nonvesicular but areolated pronotal anterior extension (Fig. 1 AEP). The narrow paranotal expansions (Fig. 1 PS) folded backwards, the proscutellum extending upto the third abdominal segment, lateral carinations absent, the median carination not reaching the tip of the posterior extension of the heavily areolated scutellum, the discoidal area divided by transverse elevated areolated, dark ridge (Fig. 1 TR), the proscutum of the male darker than that of the female, the costal margin uniserially areolated with dark transverse stripes, hemielytral margins without spines and extending 0.25 mm beyond the tip of the abdomen.

COPULATION AND EGG LAYING

Copulation takes place in the early hours of the morning and the evening but never in mid-day. The process lasts for about half an hour to one hour

The eggs are inserted into the substance of and along the length of the three major veins on the under surface of the leaf. The indented margin of the egg faces upwards with the operculum flushed with the surface of the vein. Each vein carries three to twelve eggs The under surface of the blade of the leaf is also occasionally used for egg laying but never the upper surface No smearing of faecal matter on the operculum as reported by Johnson (1936) in the case of *Leptobrysa rhododendri* and by the author in the case of *Dictyla Sufflata* has been observed here The eggs are glued to the substance of the vein by the mucilage of the leaf which later turns hard and brownish holding the egg firmly in position. Eggs under such conditions have more tough chorion and are very difficult to remove from the plant tissue without damage

STRUCTURE OF THE EGG (FIGS 7-8)

Length 0 4 mm, width 0 17 mm

The egg is divisible into three distinct regions—the operculum or cap the neck, and the body

The operculum (OP) is elliptical very dark and marked by hexagonal sculptured areas (HS) which are rather well pronounced on the inner surface It is about 0 11 mm long and 0 06 mm broad and rests on the spongy rim of the neck. The operculum falls off with a portion of the spongy tissue (SB) bordering it (sealing bar) at the time of hatching No spongy crest over the operculum as observed by the author in the eggs of *Cadmilos retiarius* has been noticed here.

The neck region is laterally compressed. The chorionic rim is continuous with the spongy region lying above it holding the operculum This spongy region is heavily porous at the distal rim (PD) but compact at the proximal area (SR) (' Problematic structureless tissue Roonwal, 1952) bordering the chorion. This compact region bears about twelve vertical canals (MC) the outlines of which are faintly marked at the porous distal rim as well as at the porous rim (sealing bar) bordering the operculum. These canals open indepen dently into a transverse narrow space (NS) just below the chorionic rim. A more or less similar structure is described by Southwood (1956) in the case of *Rhinocoris*. No branching of these canals below the level of the chorionic rim is noticed as described by Roonwal (1952) and Johnson (1936) in the case of *Telenomus Scrupulosa* and *Leptobrysa rhododendri* respectively

Beament (1947) recognized in the egg of *Rhodnius prolixus* two types of micropylar canals the pseudomicropyles with no definite external openings and the true micropyles with both external and internal openings the former are concerned with respiration while the latter with the conveyance of sperms Southwood (1956) placed Tingids eggs among those with true micropyles (cimicomorpha) and stated that the problem of respiration in this group is un solved. But as the external openings of these canals are not distinct and their out lines being faintly marked at the porous distal rim of the spongy regions and

the sealing bar of the operculum, they could be well identified with the pseudo-micropyles comparable to the "Leuckart s canals" responsible for respiration.

The body of the egg (B) is rather rough with bluntly rounded posterior end which facilitates insertion into the plant tissue. Outlines of the hexagonal areas (sculpturations) and the indented margin (IA) of the egg are heavily pigmented. According to Beament (1946) the hexagonal sculpturations are caused by the follicle cells during the secretion of the chorionic membranes. Southwood (1956) found that the shallow follicular pits would produce hexagonal sculpturations while the deeper pits impart punctate appearance to the chorion Not all eggs of *Membracidae* show sculpturations. In glycerin mounts the pigmentation fades away after some time.

IMMATURE STAGES (FIG. 9 A F)

First instar—Length 0·45 mm width 0 18 mm.

Head—Length 0.1 mm, width across the eyes 0 1 mm with a knob like anteclypeus slightly visible from above prominent post clypeus with a pair of median short tuberculated, globulated spines a similar spine on either side, towards the base of the anteclypeus and on the vertex at the base of each eye The eyes with five red, rather widely separated ommatidia. Antenna 0 17 mm long four segmented the scape and the pedicel of equal length and the shortest third segment with three or four long globulated spines the terminal segment capitate, a little longer than the 3rd with a tuft of short nonglobulated spines at the apex and four to six long slender globulated spines along its length antennal tubercle not formed. Rostrum stout with four distinct segments, reaching up to the third abdominal segment. The powerful stylets (ST) extend a little beyond the tip of the rostrum. Labrum (LB) short and broadly triangular and invisible from above. Bucculae undeveloped The anterior arms of the Y-shaped streak along which splitting of larval cuticle takes place extend from the base of the post-clypeus to the base of antennae while the median arm extends up to the second abdominal segment

*Thorax—Prothorax half the size of the entire thorax, with a globu*lated spine mounted on short tubercle on either side of the dorso-median streak a similar spine on the mesothorax on either side of the dorsomedian streak meso-and metathorax of equal size each thoracic segment *bears on* its outer margin a short tuberculated globulated spine. The legs of equal size with illmarked tarsal joints but with powerful claws femur and tibia of equal length with short nonglobulated spines along their lengths.

Abdomen—Ten segments visible first nine segments, each with short tuberculated globulated *spines on the outer margins* tenth segment with short nonglobulated spines fringing the posterior margin the second, fifth and eighth segments with a couple of light brown padded short tuberculated, globulated sp nes on the do so-median *line the pads* bearing very minute scoli the dorsal scent gland openings invisible.

Second instar—Length 0 65 mm width 0 34 mm

Head—Length 0 18 mm, width across the eyes 0.22 mm, length of antenna 0.24 mm. The pronotum covering about half the vertex, and the exposed area bearing very short scoli the tubercles more elongated and brownish the median tubercles of the post clypeus widely separated the conical projection of the anteclypeus with a couple of slender nonglobulated spines on its outer margin the tubercles of the vertex with an additional globulated spine at the base The terminal antennal segment longer than the third segment and more spinous. The rostrum with brownish terminal segment reaches up to the first abdominal segment, the coiled spring like stylet sacs extend up to the third abdominal segment. Bucculae not developed Eyes with eight to nine red ommatidia separated from each other by narrow inter ommatidial space.

Thorax—Prothorax as long as the meso-and metathorax combined with the addition of a short tuberculated globulated spine anterior to the first dorso-median tubercles slightly convex posteriorly and concave anteriorly another short tuberculated globulated spine added in front of the lateral marginal tubercle of the pro and mesothorax the dorso median tubercles of the mesothorax more elongated with padded base bearing short brownish scoli meso and metathorax of equal size, metathorax slightly concave posteriorly wingpads not developed

Abdomen—The lateral marginal tubercles more elongated with a few short scoli at their bases giving a serrated appearance. The eleventh segment telescoped into the tenth segment and attached to the tip of the rectum (anus) and protrudes out during defaecation The dorso-median tubercles to the fifth segment with more stout and dark pads than those of the second and eighth segments, the pads being studded with short brown scoli The dorsal scent glands *open between the third and fourth and fourth and fifth segments.*

Dorsally the body covered with short brownish scoli in addition to two or three more elongated slender scoli on each segment usually fringing the posterior margin.

Third instar- *Length 0 72 mm, width 0 43 mm.*

Head—Length 0·19 mm. width 0.24 mm. The median tubercles of the post clypeus meet at the base with a common pad covered with dark brown short scoli (BT) the bases of the other tubercles also of similar pads the anteclypeus partially covered by the pads of the tubercles lying on either side of it the tubercles of the vertex appear biramus by the elongation of the short tubercle added to it in the second instar Eyes with twelve to fifteen compact ommatidia. More than two-third of the vertex covered by the pronotum the scoli of the exposed region darker The third and terminal antennal segments subequal, with more globulated and nonglobulated spines. The terminal segment of the rostrum dark.

Thorax—Prothorax, convex posteriorly covering the anterior median half of the mesothorax mesothorax broader than the prothorax, concave posteriorly about ten elongated slender scoli along the posterior margins of the meso and metathorax another short tuberculated globulated spine added in front of the second pair of the tubercles on the lateral margin of the pro and mesothorax. The pads of the dorsomedian tubercles of the pro and mesothorax become darker with additional short scoli Wing pads appear as short backward extensions from the postero-lateral margin of the mesothorax covering the anterior half of the lateral margin of the metathorax.

Abdomen—Short tuberculated, globulated spine added at the bases of the outer marginal tubercles of the fourth to the ninth segments. The tubercules with more dark scoli along the length

Fourth instar—Length 1 06 mm width 0.49 mm.

Head—Length 0.25 mm width 0.37 mm rectangular postclypeus with two or three slender globulated spines on the padded bases of the median tuburcles the tubercles on either side of the anteclypeus also with a pair of slender globulated, outwardly pointing spines. Antenna 0.39 mm long third segment about half the length of the entire antenna with additional globulated and nonglobulated spines along its length antennal tubercles appear as marginal minute knobs at the base of the scape. Eyes with about twenty five compact ommatidia. The bucculae appear as thin ridges along the margin of the labiofossa but do not extend beyond the level of anteclypeus The rostrum extends up to the metacoxa

Thorax—Prothorax acutely convex posteriorly covering the whole of mesonotum outer margin widened beyond the level of the eyes two extra short tuberculated globulated spines added in front and behind the last formed tubercles of the outer margin of the pro and mesothorax mesothorax concave posteriorly as broad as the prothorax the wing pads (WP) extending upto the second abdominal segment exposing the mid region of the metathorax which bears only one lateral marginal tubercle.

Abdomen—The first segment indistinguishable, more or less fused with the thorax the conical tenth segment with additional short, tuberculated spines fringing the margin and without visible sternite The paired dorsomedian tubercles of the second fifth and eighth segments united at the base by thick pads with many dark, short scoli and a pair of long slender globulated spines the outer marginal tubercles elongated and darker and those of the ninth segment shifted dorsally posterior margins of the segment fringed with additional slender elongated scoli the submarginal area of each segment indented showing dark markings of the attachment of sternopleural muscles.

Fifth instar—Length 1 43 mm, width 0 79 mm.

Head—Length 0 34 mm, width 0.35 mm square anteclypeus visible from above with a couple of long slender globulated spines and a few short nonglobula

ted spines in-between the median biramus tubercle of the postclypeus develops a short tuberculated, globulated spine in between the rami and another pair of similar spines along the length of each ramus additional slender long scoli added on the exposed region of the head and a pair of globulated spines on the tubercles of the vertex antennal tubercles well pronounced and slightly curved at the tip. Antenna 0 47 mm long third segment a little more than half the length of the entire antenna the terminal segment as long as the first and second combined. Buccula well formed but does not extend beyond the level of the anteclypeus.

Thorax—Acutely convex posterior margin of the prothorax extends up to the anterior half of the metathorax. Slender long globulated spines added in between the older tubercles of the lateral margins of the pro-and meso-thorax. The wing pads, with a few short and long scoli extend up to the anterior half of the fifth abdominal segment leaving the mid region of the segments exposed Legs of equal size with prominent tibial spurs.

Abdomen—Same as in the fourth instar with darker pads and stouter elongated tubercles. The openings of the dorsal scent glands wider

General Remarks on Immature Stages

All the instars, when emerged are orange red, turning gradually to brown and finally to black. At the time of hatching the nymph is hyaline. The cuticular structures except the nonglobulated spines bear a sticky substance at the tip in the form of globule. The globular nature of the globulated spines is visible even after the sticky substance is dissolved in alcohol In newly emerged nymphs the amount of fluid is insignificant but a short time after it has started feeding the quantity increases and the whole cuticle shines This fluid becomes more sticky in the castings (ecdysial cuticle) Foreign dust particles stick to this fluid and camouflage the nymphs. The function of this waxy fluid is probably excretory

Patel and Kulkarney (1955) used the term 'scoli and tubercles for the same cuticular outgrowths and Roonwal (1952) used the term "lobular prominences' for the same structures. Crosby and Hadley (1915) employed the term "tubercle" for the structures on which are mounted the spines and the term "hairs' for the other simple outgrowths. The author prefers the term "scoli" for those prominences which do not bear a spine atop to distinguish them from the more pronounced padded and nonpadded structures, (tubercles) at the tip of which are mounted the globulated spines According to this the cuticular structures are classified as follows (Fig 10 a-g)

1 Short scoli with sharp tip
2. Long slender open scoli.
3 Nonglobulated spines.
4 Short tuberculated, globulated spines.
5 Long tuberculated, globulated spines.

(a) Simple serrated tubercles
(b) Biramus serrated tubercles.

The scoli arise directly from the cuticle while the tubercles always carry dermal layer and the spines are always articulated on the tubercles. The long tubercles are always studded with short scoli along their lengths imparting them a serrated appearance. The measurements of the tubercles and spines of the various instars show that the globulated spines of the first instar are longer than those of the fifth instar On the other hand the tubercles increase in length in the successive stages (Table I) The addition of new tubercles are always anterior to the older ones. The instars do not exhibit congregational tendencies as the various instars roam about the leaves and buds and the whole developmental cycle is not completed on a single leaf as it is in the case of *Urentus* Spp

Table I

Statement showing the relative lengths of the tubercles and globulated spines in the various nyphal instars

Instars	Tubercles	Spines
First	0 008 mm	0 048 mm
Second	0 058 mm	0 044 mm
Third	0 08 mm	0.044 mm
Fourth	0 08 mm	0 036 mm
Fifth	*0 1 mm*	0 036 mm

KEY TO THE NYMPHAL INSTARS OF MONOSTEIRA MINUTULA MONTANDON

1 Wing pads not developed Median tubercles of the head widely separated 2

Wing pads developed Median tubercle of the head united at the base by a common pad to appear biramus 3

2 Eyes with 5 ommatidia rostrum reaching the third abdominal segment First instar

Eyes with 8-10 ommatidia rostrum reaching not beyond the second abdominal segment Second instar

3 Wing pads do not extend beyond the first abdominal segment eyes with not more than 15 compact ommatidia prothoracic margin not acutely convex Third instar

Wing pads extending beyond the first abdominal segment; eyes with more than 20 compact ommatidia prothoracic posterior margin acutely convex 4

4 Wing pads rounded, extending upto the lateral half of the second abdominal segment. Fourth instar

Wing pads elongated reaching up to the fifth abdominal segment Fifth instar

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Summary

Monosteira minutula Montandon a sap sucker on *Zizyphus jujuba* is recorded here for the first time from the Oriental region. It is a summer species spreading from March to October The damage caused to the tree is not very serious as the population is usually thin. The structure of the egg and the immature stages have been described in detail and the cuticular structures classified.

References

1 Beament, J W L. 1946. The formation and structure of the chorion of the egg of Heteropteran *Rhodnius prolixus* *Quart. J Micr. Sci.* 87 393-439.

2 Beament, J W L. 1947 The formation and structure of the micropylar complex in the egg shell of *Rhodnius prolixus* tal (Heteroptera Reduviidae) *J Exp. Biol.* 23 : 213-33

3 Crosby C R. & Hadley 1915. On the Rhododendron Lace bug, *Leptobyrsa explanata* Heid *J Econ. Ent.* 8 409-414

4 Distant, W L. 1903 *The Fauna of British India including Ceylon & Burma.* Rhyn-chota 2 (1) (Heteroptera) 122 144

5 Distant, W L. 1910. *Ibid.* Rhynchota 5 (Heteroptera, Appendix) 100-126.

6 Drake C. J & F A. Ruhoff. 1960 Lace bug genera of the world *Proc U S Nat. Mus* 112 (3431) 1 105

7 Heidemann, O. 1911 Some remarks on the eggs of North American species of Hemiptera Heteroptera. *Proc. Ent. Soc. Wash.,* 13 128-140

8 Johnson C. G 1936 The biology of *Leptobyrsa rhododendri* Horvath. *Ann. Appl Biol.* Cambridge, 23 312 368.

9 Kershaw J C. W & Kirkaldy G W 1909 Biological notes on Oriental Hemiptera. No. 3 *J Bombay Nat Hist. Soc* 19 333-336.

10 Khan, A. H 1945. On the Lantana Bug *Teleonemia scrupulosa* (Stål). *Indian J Ent.,* 6 (1-2) 149-161

11 Kirkaldy G W 1902 Memoirs on Oriental Rhynchota. *J Bombay Nat. Hist. Soc.* 14 297-299

12 Lawson F A 1959 Identification of the nymphs of common families of Hemiptera. *J Kansas Ent. Soc* 32 (2) 88-92

13 Leston, D 1953. The eggs of Tingidae especially *Acalypta parvula*. *Ent. Mon. Mag. London*, 89 : 132 134

14 Leston D J C. Pendergrast & T R. E. Southwood 1954 Classification of Terrestrial Heteroptera (Geocorisae) *Nature* London, 174 : 91 93.

15 Livingstone, D. 1959 On the bionomics and immature stages of *Urentius euonymus* Dist. the Hollyhock Lacebug (Heteroptera Tingidae) First All India Congress of Zoology abstracts, pp. 46-47

16 Livingstone, D 1960 On the incidence of the Tingid population in Agra with a note on the distribution of *Teleonemia scrupulosa* Stål. *Agra Univ J Res* (Sci) X (2) 187 192.

17 Mathur R. N 1955 Immature stages of *Tingis beesoni* Drake. *Entomologist*, London 88 (1110) 248-251

18 Menon M. G R. & S. A. Hakk 1959a. Some tingids of economic importance from Delhi State. *Proc 46th Indian Sci. Cong.*, (3) : 392 393

19 Menon, M G R. & S A. Hakk 1959b. A revision of the genus *Urentius* Dist. with descriptions of five new species (Heteroptera Tingidae) *Ibid.*, (3) : 391

20 Montandon A. L. 1897 Nouvelles espèces d Hemipteres Heteropteres d Algerie et de Tunisie *Rev Ent. Franc* pp. 97 101

21 Morill, A. W 1903 Notes on the immature stages of some Tingids of the genus *Corythuca*. *Psyche* 10 127 134

22 Patel, R. C. & H L. Kulkarney 1955. Bionomics of *Urentius echinus* Dist. (Hemiptera: Heteroptera Tingidae) as important pest of Brinjal (*Solanum melongena*) in th North Gujarat *J Bombay Nat. Hist. Soc* 53 (1) 86-96.

23 Pendergrast, J G. 1959. Studies on the reproductive organs f the Heteroptera with a consideration of their bearing on their classification. *Trans. R. ent. Soc. Lond* 109 (1) 1-63.

24 Roonwal M. L. 1952. The natural establishment and dispersal of an imported pest in India, the Lantana bug (*Teleonemia scrupulosa* Stål) (*Lantana* Dist.) (Hemiptera Tingidae) with description of the eggs, nymphs and adult. *J Zool. Soc India*, 4 (1) 1 16.

25 Samuel, C. K. 1939 Oviposition of the Tingid *Monanthia globulifera* Walk. *Indian J Ent* 1 (3) 93-99

26 Sharga, U S. 1955. Bionomics of *Monanthia globulifera* Walk (Hemiptera Heteroptera Tingidae) *J Bombay Nat Hist. Soc* 51 (4) 885-889

27 Sinha S. S. 1931 Some observations on the attack of *Urentius echinus* Dist. on Brinjal at Sabey Farm *Indian J Ent.* 13 (2) 182.

28 Southwood, T R E. 1956. The structure of the eggs of Terrestrial Heteroptera and its relationship to the classification of the group. *Trans R Ent Soc Lond* 108 (6) 163-221

29 Southwood, T R. E. & G. G. E. Scudder 1956. The bionomics and immature stages of the Thistle lace bugs (*Tingis ampliata* H S. and T cardui L. Hemiptera Tingidae) *Trans. Soc. Brit Ent.* 12 (3) 93-112

30 W s, H B. & E. L. Dickerson 1918a. The early stages of *Corythucha pergandei* Heid. *Ent. News*, 29 205.

31 W s, H B. & E. L. Dickerson 1918b. Life history and arly stages of *Corythucha parshleyi* Gibson *Can. Ent.* 50 401 406.

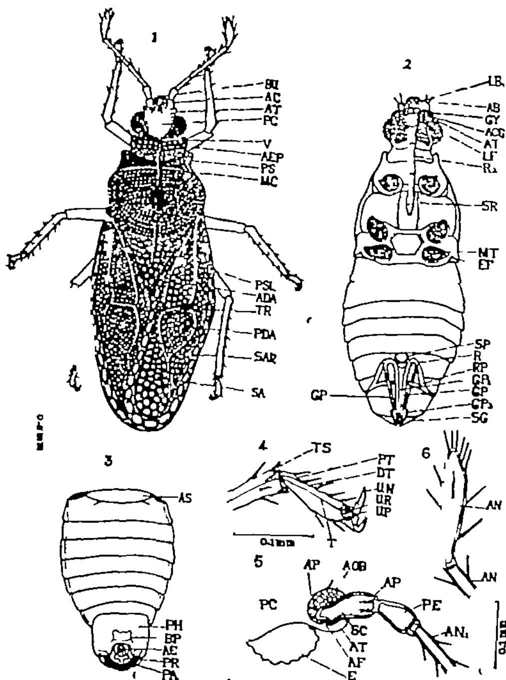


Fig 1 *Monosteira minutula* Montandon, adult—AC. anteclypeus ; ADA. anterior discoidal area ; AEP anterior extension of pronotum ; AT antennal tubercle ; BU buccula ; MC. median carination PS. paranotal fold ; PSL. proscutellum ; SA. sutural area SAR. subcostal area ; TR. transverse ridge ; V vertex.

Fig 2. *Ventral view of the female with genitalia exposed*—AB. anterior extension of buccula ; AOB. anterior outer lobe of buccula ; AT antennal tubercle ; EF epimeral fold ; GP gonocoxa (second gonocoxopodite) ; GP_1 first gonapophysis ; GP_2 second gonapophysis ;

GP_3 third gonapophysis (gonoplac) ; GY gymnoid ; LB. labiofossa ; MT meatal cavity ; R_2 second rostral segment ; R. ramus RP ramal plate (gonangulum) ; SG_{10} tenth segment of the abdomen SP subgenital plate ; SR. sternal ridge.

Fig 3. *Dorsal view of the abdomen of the male with the genitalia exposed*—AE. phallus ; AS_1 first abdominal tergite BP basal plate PA. paramere ; PH pygophore ; FR. suspensory arm.

Fig 4. *Distal region of the leg*—DT distal tarsus PT proximal tarsus ; T tendon of the retractor of unguis TS. tibial spur ; UN Unguis ; UR. Unguitractor ; UP unguifer process of tarsus.

Fig 5 *Basal segments of the antenna with the antennal socket*—AF antennifer (articular knob or pivot) ; AN_1 first annulus of the flagellum ; AOB. anterior outer lobe of buccula ; AP_1 apodemes for the levators and depressors of scape ; AP_2 apodemes for the levators and depressors of the pedicel ; AT antennal tubercle ; E. eye ; PC. postclypeus PE. pedicel; SC. scape.

Fig. 6. *Flagellum*—AN_1 first annulus AN_2 second annulus.

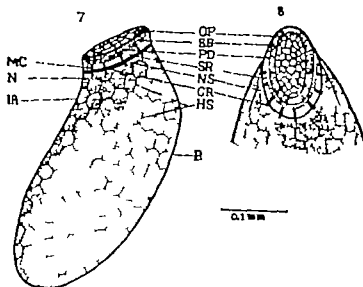


Fig 7 *Lateral view of the egg* ; Fig. 8. *Opercular view of the egg*—B. Body ; CR. chorionic rim HS. hexagonal sculpturations ; IA. indented margin ; MC. micropylar ertical canals (Leuckart canals) ; N neck ; NS. narrow transverse space ; OP operculum; PD. porous distal rim of the spongy region of the neck ; SB. sealing bar

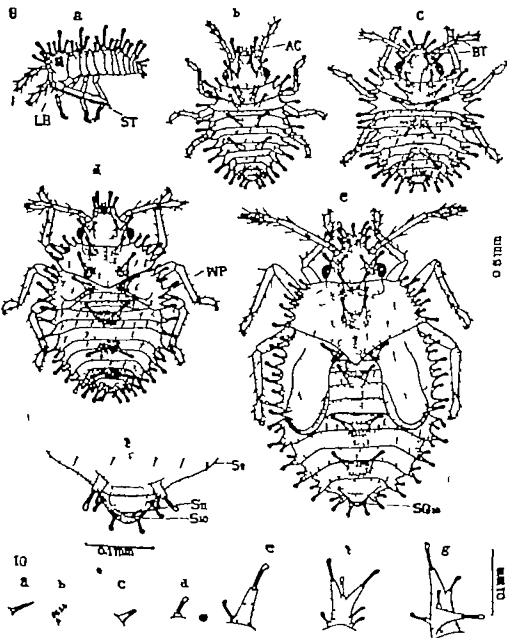


Fig. 9 *Immature stages*—a. 1st instar (lateral view); b. second instar; c. third instar; d. fourth instar; e. fifth instar; f. terminal abdominal segments of fifth instar; AC anteclypeus; BT biramous tubercle of the postclypeus; LB. labrum; S_9, S_{10}, S_{11} ninth, tenth and eleventh segments of the abdomen; WP wing pad.

Fig. 10 *Cuticular structures of the last instar nymph*—a. nonglobulated spine; b. short sharp scoli; c. long, slender scolus; d. short tuberculate, globulated spine; e. long, serrated tubercle with globulated spine atop; f. biramous tubercle; g. tubercle at the base of the eye on the vertex.

EXTERNAL MORPHOLOGY OF THE HEAD CAPSULE OF *SPHRACEPHALA HEARSEIANA* WESTW (DIOPSIDAE DIPTERA)*

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INTRODUCTION

The Diopsid flies have been neglected by morphologists, while taxonomy has received some attention from systematists like Curran (1934) Chen (1949) Ouchi (1951) and Seguy (1949 and 1955) This paper summarizes the results of our studies on the morphology of the head capsule. Accounts of other systems will follow in subsequent papers.

MATERIAL AND METHOD

The flies for the work, collected from the wall s of bath room, Lloyd s hostel, (St. John s College Agra) during the months of November and December were treated with 10% cold KOH for 24 hours neutralised in acetic acid, upgraded and preserved in glycerine. During August September the flies are abundant on the leaves of *Ageratum* sp. (Compositae) Dissections were made in canada balsam for detailed study and diagrams were drawn with the help of camera lucida.

THE HEAD

The head is hypognathous produced laterally into long eye stalks, bearing compound eyes terminally The proboscis is attached to the oral rim by a membranous area and remains folded when not in use. The ommatidea impart a reddish tinge to the eyes. The head is 0 62 mm long and 2.12 mm wide in male and 0.50 mm long and 1.62 mm wide in the female. The cranium is a compact capsule and the obliteration of the sutures has rendered the demarcation of various areas extremely difficult.

Sutures and Sclerites —(Figs. 1 and 2) Dorsally the vertex (VTX) bears a sclerotised circular area the ocellar plate (OCP) bearing three ocelli arranged in a triangle the lateral ocelli (LOC) above and the median ocellus (MOC) below Below the vertex on the anterior side the whole area stretching inbetween the compound eyes (CE) represents the frons (FRS) which is divided into the 'post frons' (POFRS) and the pre frons (PFRS) by a thick ptilinal suture (PTS) The ptilinal suture, which is ∩-shaped with its arms over th bases of the antennae in typical cyclorrhaphous Diptera has almost become

*Contribution No. 94 from the School of Entomology St. John College, Agra

straightened out with the arms extending over the antennal sockets (ANTS) and terminating anteriorly at the bases of the eyes. The post frons (POFRS) stretches from the ptilinal suture (PTS) to the oral rim while the region above the suture upto the vertex forms the pre frons (PFRS) Ferris (1950) and Nayar (1961) described similar areas in *Drosophila* and *Dacus* respectively The post frons (POFRS) bears the pair of antennae far apart from each other almost touching the limbs of ptilinal suture thereby obliterating the frontal lunule. The cylindrical eye stalks (ES) of Lefroy (1909) are probably constituted by the lateral extentions of the frons, vertex and genal region. The inverted V-shaped clypeus (CLY) is separated from the cranium by the transverse membrane on the anterior border of the oral rim.

Posteriorly the occipital foramen (OCCF) is bounded dorso-laterally by a distinct post occipital suture (POCCS) with long posterior tentorial pits (PTP) in its lateral limbs Internally this suture forms a very well-developed strong post-occipital ridge (POCCR) forming broad apodemal plates laterally for the attachment of dorsal longitudinal prothoracic muscles The well-marked post-occiput (POCP) is produced into a pair of small prominent processes, the occipital condyles (OCCC) for articulation with the cervical sclerites. In addition to these condyles, the post occiput bears dorsally a prominent highly sclerotised knob-like structure. Dorsal to the post-occiput, there is present an indistinct ridge internally probably marking the position of occipital suture which has become absolutely lost The area between the post-occipital suture and this ridge may well be considered as representing the occiput, the absence of occipital suture converts the whole posterior region into a complex of occipito-postgenal hypostomal area bearing the occipital foramen in the middle. An identical fusion of the areas has been described in bee's head Snodgrass (1935) and reported in other higher Diptera The ventral border of the occipital foramen is constituted by the transverse hypostomal bridge (HSB) Internally it is supported by a transverse sclerotised plate, the corpotentorium (CORPT) formed by the fusion of the posterior-tentorial arms arising from the posterior tentorial pits (PTP)

The tentorium.—(Fig 2) Tentorium is extremly reduced and is represented only by the tentorial bridge or corpotentorium (CORPT) formed by the fusion of the posterior tentorial arms constituting a support to the ventral part of the rim of the occipital foramen

THE CHAETOTAXY

Inner verticals—(Figs. 1 and 2 IV) A pair of long bristles on the vertex, a little away from the ocellar plate, one on each side

Outer verticals—(Figs. 1 and 2 OV) A pair of bristles nearly half the size of the inner verticals near the bases of the eyes.

THE HEAD APPE[illegible]

1 *The Antenna.*—(Fig 9) The an[illegible] are brown pubescent and three segmented The base of the ant[illegible] articulated to the antennal socket (ANTS) by an antennifer (ANT) The scape (SC) or the basal segment is smallest, bearing four term[illegible] second antennal segment or the pedicel (PED) is little longer than the scape narrow proximally and thick distally with the distal end nearl[illegible] three times as thick as base, bearing ten prominent bristles apicall[illegible] The third antennal segment or the flagellum (FLG) is nearly as long as wide with two segmented dorsal arista (ART) arising subapically The proximal segment of the arista is very small and the second is extremely long and thread-like

2. *Mouth parts*—(Figs. 3 4 5 6 7 and 8) The mouth parts are the typical non-piercing muscoid type, comprising a composite structure the proboscis, made up of labrum-epipharynx (LABE) and labium hypopharynx (LABH) The mandibles are absent while the maxillae are represented only by a single segmented maxillary palp The proboscis consists of the following three parts —

(i) A large basiproboscis or rostrum.

(ii) A mediproboscis or haustellum

(iii) The distiproboscis or labella or lobes of the so called "oral-sucker"

(i) *Basiproboscis or Rostrum.*—It represents the basal membranous portion of the proboscis attached to the oral rim. On its anterior side an inverted V-shaped sclerotised piece represents the clypeus (CLY) and the surrounding membranous area is the peripheral clypeal membrane (PCLYM) of Snodgrass (1944) Antero-distal to the terminal ends of the clypeal arms, arise a pair of long single segmented maxillary palps (MAXP) The maxillary palp is nearly five times as long as thick, with a sensorium (SEN) running from the middle of its length to its terminal end along the outer margin. The sensorium and the outer margin of th[illegible] palp is fringed with numerous prominent setae. The cibarial pump (CIBP) is an elongated chamber with a median ridge (MR) into which opens proximally the food canal of the proboscis. The inward lateral inflections of the clypeus, the lateral plates (LP) meet latero-ventrally the walls of the cibarial pump forming the fulcrum, for the movements of th[illegible] proboscis.

(ii) *Mediproboscis or Haustellum.*—It is the middle cylindrical portion, projecting downwards and forwards from the distal end of th[illegible] rostrum in a stretched out condition but lies folded along the anterior surface of the rostrum when not in use. The anterior surface is covered over by a long flap-like labrum-epipharynx (LABE) attached to the distal end of the rostrum, bearing a pair of internal rods, the labral-apodemal rods (APDR) proximally The inner surface of labrum-epipharynx bears two longitudinal ridges (LR)

which rest on the labium-hypopharynx (LABH) below enclosing a food canal in between them. The hypopharynx is completely fused with the inner wall of the labium. Arora (1956) and Nayar (1961) observed similarly in adult Hymenoptera and Diptera respectively The labium hypopharynx is supported posteriorly by a sclerotised plate, called theca or mentum (MNT) The mentum bears an anterior and posterior cornua (COR) The distal *cornua meets the supporting structure of labella (LAB) called the furca.*

(*iii*) *The distiprobascis* —The terminal part of the proboscis the oral disc or oral sucker or labella is in the form of an oval disc formed by the fusion of two membranous labial palps (Crampton 1923) *enclosing centrally an opening*, the oral aperture (ORA) The inner surface is traversed by canaliculi called the pseudotracheae (PSEUT) all converging at the oral aperture, while the outer surface bears the sensory bristles. The furca, supporting the labella *is a complicated structure It is constituted by two long furcal arms*, two transverse pieces attached to the former by an intermediate piece which in turn supports another longitudinal sclerite.

Summary

The head of *Sphyracephala hearseyana* Westw is hypognathous. The frons is produced into long eye stalks laterally with the compound eyes terminally The thick ∩-shaped ptilinal suture divides the area of the frons into anterior and posterior parts. The inverted V—shaped clypeus is clearly separated from the frons above. The tentorium is greatly reduced represented by the tentorial bridge only The proboscis is a typical non-piercing type.

Acknowledgements

Our sincerest thanks are due to Dr T Singh Professor of Zoology & Entomology School of Entomology St John's College Agra for his valuable guidance and facilities for work. We are extremely indebted to Dr Santokh Singh for his able suggestions and help. Our thanks are also due to the authorities of Z S I Calcutta for identifying the insects.

References

1 Arora G L. 1956. The relationship of Symphyta (Hymenoptera) to their orders of insects on the basis of adult external morphology *Res Bull. E. Panj. Univ* 90 : 85-119

2. Chen S H. 1949 Records of the Chinese Diopsidae and Celyphidae (Diptera *Sinensia Shanghai* 20 7 18.

3 Crampton, G. C 1923 A comparison of the labium in certain holometabolous insects from the standpoint of Phylogeny *Proc Ent Soc Washington*, 25 : 171 180

4 Curran C. H 1934 Note and description of African Diptera. *Amer Mus. Novit* New York No. 710

5. Ferris, G. F 1950. External morphology of *Drosophila* given in the biology of *Drosophila*. Ed ted by M. Demerec, N Y John Willy & Sons, Chapman & Hall Ltd. London.

6. Lefroy H. M. 1909. Indian insect life. Thacker Spink & Co 2 Creed Lane London.

7 Nayar J L. 1961 External morphology of the head capsule of the fruit-fly *Dacus dorsalis* Coq (Trypetidae : Diptera) *Agra Univ J Res.* (*Sci.*) 10

8. Ouchi, Y 1951 On two new species of Chinese stalked-eyed flies (Diptera Shalca, Diopsidae I) *J Shanghai. Sci Inst* (N S.) 2 45-48

9 Seguy E. 1949 Diopsides de Madagascar *Mem. Inst. Sci Madagascar Tananarive* 3A 165-170.

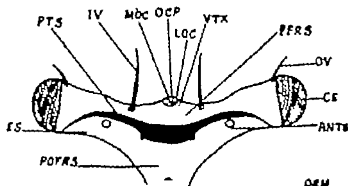
10. Seguy E. 1955. Dipteres Diopsides africains nouveaux on peu connus. *Bull Inst Franc Af Noire Dakar* (A) 17 1102 1124

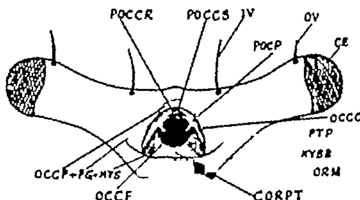
11 Snodgrass, R. E. 1935. Principles of Insect Morphology McGraw Hill Book Co New York and London.

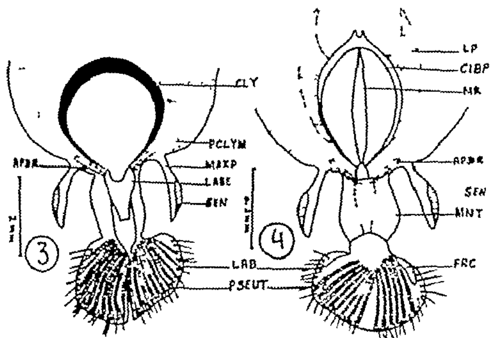
12. Snodgrass, R. E. 1944 The feeding apparatus of biting and sucking insects affecting man and animal. *Smithson. M sc Coll.* 104, No. 7

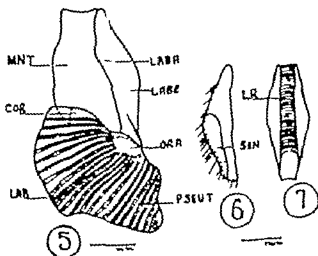
1 Head (anterior view) 2. Head (posterior view)

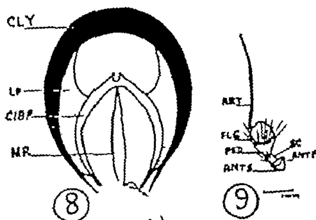
ANTS antennal socket ; CE compound eye CORPT corpotentorium ES. eye-stalk ; HYSB. hypostomal bridge IV inner vertical bristle LOC. lateral ocellus ; MOC. median ocellus ; OCCC. occipital condyle ; OCCF occipital foramen ; OCP ocellar plate ; OCCP occiput ; ORM. oral rim ; OV outer vertical bristle OCCP+PG+HYS. occiput+postgena+hypostome POCP post occiput ; POCCR. post occipital ridge ; POCCS post occipital suture ; PFRS. post frons PTP posterior tentorial pit PTS. ptilinal suture ; VTX. vertex






CLY
PCLYM
APDR
MAXP
LABE
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CIBP
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APDR
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MNT
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MNT
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CLY
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5. Proboscis (lateral view) 6. Maxillary palp. 7 Labrum-epipharynx. 8. Fulcrum and cibarial pump. 9 Antenna.

ANTF antennifer ; ANTS antennal socket ; ART arista ; CIBP cibarial pump ; CLY clypeus ; COR. cornua ; FLG flagellum LAB labella ; LABE labrum-epipharynx ; LABH. labium-hypopharynx LP lateral plate LR. longitudinal ridge ; MNT mentum MR. median ridge ORA. oral aperture ; PED pedicel ; PSEUT pseudotracheae SC. scape ; SEN sensorium

CORRELATION OF CHOLESTEROL CONTENT OF AORTIC WALL WITH GROSS HUMAN ATHEROSCLEROSIS

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SUMMARY

Aortas from 100 medico-legal necropsies, of persons ranging in age between 1 and 73 years were examined for the amount and severity of atherosclerosis by the method recommended by Tejada and Gore (1957). Total cholesterol contents of the intima and media of the whole aorta was determined in all the cases by Reinhold and Shiel's modification of Myer and Wardell's Method (1936). Blood total cholesterol was estimated in 90 cases by the method of Zak, Dickman and White (1954). An attempt was made to compare the amount and severity of aortic atherosclerosis with that found in the coronary, cerebral, renal and pulmonary arteries and to correlate it with the total cholesterol content of the aortic wall. The total blood cholesterol levels were evaluated as an index of amount and severity of atherosclerosis as obtained in above arteries.

The atherosclerotic process increased with advance in age. The relationship between the severity of atherosclerosis and age was shown to be statistically significant after 30 years of age (r=0·8, t=13·6 and hence highly significant at 98 degrees of freedom at 5% level).

In the first decade 37.5% of the aortas were completely free of atherosclerotic lesions. 91.5% of aortas in second decade showed some lesions of atherosclerosis while all aortas had atherosclerotic lesions after the second decade.

The extent of fatty streaks (Grade I), fibrous plaques (Grade II), complicated lesion (Grade III) and calcified lesions (Grade IV) were estimated in terms of percentage of intimal surface affected by each type of lesion and the results were expressed by atherosclerotic profile and atherosclerotic index.

The fatty streaks, which were prominently displayed on gross examination by Sudan IV staining, were detected earliest at the age of 2½ years. The percentage surface area covered by fatty streaks increased from a very low mean value (0·62%) in the first decade to a mean maximum of 14·23% in the 3rd decade and then gradually declined so that by the 7th decade the fatty streaks occupied only 0·4% of surface (mean surface area involvement).

This is an abstract of the thesis submitted for the degree of Doctor of Medicine of the Agra University, Agra for the year 1960.

The fibrous plaques were seen for the first time in the second decade in the aorta. The mean percentage surface covered by fibrous plaques rose from a minimum of 0·13 per cent in the second decade to a maximum of 8 2% in the fifth decade.

Complicated lesions (Grade III) were seen for the first time in the fourth decade. The mean percentage surface involved by complicated lesions increased from 0 23 % in the fourth decade to 25 0 % in the eighth decade.

Calcified plaques in the aorta were seen for the first time during sixth decade and the mean percentage surface involved by them increased from 1 23 % in sixth decade to 10 % in the eighth decade.

Differences in the incidence and pattern of progression of aortic atherosclerosis, were noted between the two sexes While in males the mean atherosclerotic index, which is an expression of the amount and severity of atherosclerosis, increased steadily through each successive decade especially after third decade the females showed a sharp rise from a mean atherosclerotic index of 1 8 in the fifth decade to 6 75 in the sixth decade. The incidence of aortic atherosclerosis in females was much lower before the fifth decade and although it rose sharply in sixth decade it was less than that observed in the males (Mean atherosclerotic index for females in sixth decade was 6 75 while that for males was 8 475) The possible mechanism responsible for this sex difference has been discussed

A comparative study of aortic atherosclerosis as found in the present series, has been done with the reported incidence of aortic atherosclerosis in other races. The differences and their significances have been discussed.

The progression of severity of aortic atherosclerosis with age has been compared with that in coronary cerebral, renal and pulmonary arteries.

The concentrations of total cholesterol in the intima and media of whole aorta was found to be directly related to the amount and severity of atherosclerosis in the aorta and to the aging process, independently of each other The mean aortic wall cholesterol in mgms./100 gms. of fresh aorta increased from 59 75 for the first decade to a maximum of 460 in the eighth decade. When all the aortas were divided into eight arbitrary groups according to their atherosclerotic indices, the mean total cholesterol contents in mgms /100 gms. of fresh aorta, was found to be 120 17 for the lowest group and increased successively through the various groups to a maximum average of 655 7 for the eighth group As the total cholesterol contents of aortic wall may be influenced both by the aging process and atherosclerosis an attempt was made to dissociate these two factors in their influences on the concentration of total cholesterol in aortic wall For this purpose the correlation was done by finding out the coefficient of partial correlation between aortic wall cholesterol and age with disease (expressed in numbers

by means of atherosclerotic index) excluded Similarly the coefficient of partial correlation between aortic wall cholesterol and atherosclerosis with the age excluded was found out The value of former (r 12·3) was 0 313 and that of latter (13 2) was 0 21 The calculated values of 't' at 98 degrees of freedom (n=100) was calculated and they were 3 26 and 2 126 respectively These values at 5% level are highly significant. Thus the statistical analysis of our datas strongly supported the hypothesis that aortic wall total cholesterol is directly related to the amount and severity of atherosclerosis and to the agin process, irrespective of each other

The above results were obtained when the group was analysed as a whole. However marked variations were noted when individual cases were analysed.

An attempt has been made to compare the morphological and chemical methods of grading

The mean total blood cholesterol level showed a tendency to rise from youth through middle age and decline in old age. The mean total blood cholesterol for first decade was 124 29 mgm. (standard error of mean ± 13 32) while that for the fifth decade it was 170·33 (standard error of mean ± 10·87) The mean blood cholesterol levels for sixth and seventh decades were 156 87 mgms. and 167·5 mgms.% (standard error of mean ± 15 18 and ± 34·914 respectively)

No particular correlation could be observed between the total blood cholesterol levels and the amount and severity of gross atherosclerosis.

EPIDEMIOLOGICAL CLINICAL AND HISTOPATHOLOGICAL STUDIES OF CERVICAL CANCER*

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INTRODUCTION

Cancer as a scourge does not vary much in its incidence in different parts of the world But the frequency of involvement of the various sites in the body shows wide variation in different countries and communities.

The geographical pathology depends on the environmental factors, which are based on the habits, customs socio-economical conditions, psychophysical stress etc. This is well illustrated by the varying incidence of cervical cancer in defferent parts of the world. It is now well recognized that the uterine cervical cancer is the most frequent malignant tumour among the Indian women (14 19) Besides it also affects the younger age group in India as compared to England or America.[11] Most of the cases here belong to higher malignancy grades.

The problem of cancer cervix in India is therefore of high incidence, low age group and higher grade of malignancy

MATERIAL AND METHODS

The material for the epidemiological and clinicopathological studies of cancer cervix was obtained from the past 10 years (from 1949 to 1958) record of the Pathology Department of S N Medical College, Agra and the recent cases were collected from the Gynaecological ward and Radium Institute. In total 937 cases with their available histories and biopsies were studied.

OBSERVATIONS

Incidence The incidence of malignant tumours of different parts of the body which have been received in the Surgical Pathology Laboratory from January 1949 to June 1959 have been analysed. It shows among the total 3656 malignant cases, cervical cancer was seen in 937 (25 62%) patients. Its incidence comes next to intraoral cancer (30 30 %) Among the cancer of the female genital tract it occupies 85·02% of case and of this epidermoid carcinoma is 92.10% and adenocarcinoma 1.38%.

Age incidence 40.90% of cases were within 31-40 years of age and 56.71% of cases, cancer of th cervix occurred between 21-40 years. The

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* This is an abstract of the thesis submitted for the degree of Doctor of Medicine (P thology) of Agra University Agra for the year 1960.

Medical College, Agra, cancer cervix covers 25 62 per cent of all malignan tumours. In the present series frequency of cervical cancer among all the carcinomas of female genital tract, is 85 02 per cent This finding adds more gravity to the problem of high incidence of cervical cancer in India

Among the observations of western and Indian workers, the peak age shows considerable variation. The present study shows the maximum incidence is between 36 to 40 years (27 76%) and 45 8 years is the average age of the patients. In fourth decade of life the incidence is found to be 40.90% and within 21-40 years it occurs in 57 71% of cases Fifth decade shows 29 05% of incidence. These figures signify that cervical cancer in this part of the country shows predilection for the 4th decade of life and 5th decade comes next. These findings are quite significant, as Ewing's[6] observation was that the tumour was prevalent in 5th decade, Palma[14] observed that half of the cases occurred in 40-55 years of age. From Guntur Rao *et al*[17] reported that the peak age was 31 50 years.

The average age of intraepithelial carcinoma in the present series is 38.2 years which is 7 6 years lower than the invasive type. Massey *et al*[18] who found that the average age of intraepithelial cancer cases was 43.3 years and in invasive group it was 49 years. The difference was only 5 6 years.

In the present study the average age incidence of 13 cases with adenocarcinoma is 36 13 years which is 9 67 years less than that of epidermoid carcinoma. Cancer of the uterine cervix is predominantly a disease of the married life, showing predilection for woman who have born children. It is said 1 18 that early marriage leads to excessive active sexual life so the immature cervical epithelium is easily traumatised and an instability is set up there. This instability associated with chronic cervicitis leads to hyperplasia and disturb the epithelial equilibrium at the squamocolumnar junction. The average age of marriage in the present series is 14 42 years which is significantly low as compared to that of other countries Rewell[19] emphasised that the incidence of cervical cancer at an early age in Madras was due to an early marriage, which was in an average 14 2 years, whereas in Britain it was 23 years

Homburger[9] feels that the lower incidence of cervical cancer among the nuns is not due to the abstinence from sexual intercourse, but may be due to the absence of pregnancy which might have some stimulating effect Though it is not acceptable to many but some amount of trauma and super-added infection do occur irrespective of the degree of normalcy of the delivery Hence one normal delivery may bring predisposing factors to play

The mean age at first child birth, in the present series is 17 32 years which is quite low in comparison to the figures of other countries. But this

study has failed to establish association between the number of pregnancies and supervention of cancer Most of these patients had large number of children In an average it was 6 7

Chronic cervicitis is a factor which helps in carcinogenesis with some unknown adjuvants. Cashman[3] and Gagnon[7] proved that control of cervicitis would check the incidence of cervical carcinoma. This view is opposed by McKelvey[13] Professor Wahi thinks that no adult cervix is free from infection. Considering all these points excessive importance can not be put on this common factor

Ayre[1] believes that high concentration of oestrogen either general or locally in the cervix has got important role in cervical carcinogenesis Though there is not much support in favour of this theory

It is the general opinion of many workers[4 5 8 17] in various countries that cervical cancer is an intimate associate of poverty This study also shows that majority of the patients have got very low income as compared to the number of dependants

Recently considerable emphasis has been put on the psychophysical stress, which is considered as an important factor[10] in the pathogenesis of cervical cancer Jones thinks that psychophysical stress and poverty are the only factors which are constantly present in cancer cervix cases

It is well known fact that early stage of cervical cancer is symptomless and this mute phase is the main problem of its early ditection. When the disease produces symptoms it is well advanced. In the present observation 67 83% of cases attended hospital within 6 months of the onset of symptoms *and 84 68 % cases were clinically stage II and III The number of patients in* stage III is more than that of stage II These findings drive one thing in mind is that by studying the patients in the hospital alone it is not possible to get the cases in an early stage.

Warren[20] studied 102 cases of epidermoid carcinoma of uterine cervix, in which grade III was found only in 18 patients and grade II claimed highest number Kristner *et al*[12] also reported grade II as predominating one. The present series shows an alarming high proportion of grade III cases (57.52%)

The problem of higher grade of malignancy in the present series of cases can not be answered on the basis of lower age incidence Some other factors are to be found out to explain this observation.

Conclusion and Summary

Analysis of all cancer cases from the record of the Pathology Department S. N Medical College, Agra shows the incidence of cervical cancer is 5 6% and of all malignant tumours of the female genital tract it is 85 02%. Intra

epithelial carcinoma has been diagonised in 4.58 % of cases Maximum in cidence is in 4th decade Age of marriage and age of first child birth show some significant role.

The theory of oestrogen effect as a major cause could not be supported from the present study

From the study of socio-economical conditions it is seen that cervical cancer is more in poor class of people.

Clinically 81.68 % of cases were in the stage II and III

67 82 % of cases attended hospital within 6 months of the onset of symptoms

Histologically 57.52 % of the tumours belong to ep dermoid carc noma grade III Relationship between the histological grades and age incidence could not be established.

References

1 Ayre J E. Am. J Obst. Gynec; 54: 363, 1947
2. Bose E. J J Obst. & Gynec Ind, 1 1 1950.
3 Gamman, B. Z Am. J Obst. & Gynec 45 190 1945.
4 Clemmesen J J Nat. Cancer Inst, 12 1 1951 52
5. Devore, P F Bull. T c. N t. Hyg., 6: 573 1951
6. Ewing, J. Neoplastic diseases, 4th Edition.
7 Gagnon P Am. J Obst. & Gynec., 60 516 1950.
8. Hein, H. C. et al. Ibid 76 726, 1958
9. Homburger, F The biologic basis of Cancer management, New York. 1957
10. Jones, E. G. et al. Am. J Obst. & Gynec, 76: 1 1958.
11. Khanolkar V R. Acta Unio. I er Nat. Contra Cancrum, 6: 881 1948-50
12. Kottmeier, R. W et al. Am. J Obst. & Gynec, 61 1283 1951
13. McKelvey J L. Quoted by 21
14. Mitra, S J Obst. & Gynec. Ind., 7 1 1957
15 Murphy et al Am. J Obst. & Gynec 77 957 1959
16. Palma, S D Am J Surg 79 793, 1950
17 Rao S S, et al. J Ind. Med. Ass, 33 463 1959
18. Rewell, R. E. J Obst. & Gynec. Brit. Emp. 64 821 1957
19. Wani, P N et al. J Ind. Med. Ass. 31 309 1958.
20. Warren S. Arch. Path. 12: 783 1931
21 Wynder, E.L. et al. Am. J Obst & Gynec., 68 1016, 1954

HISTOLOGICAL EVALUATION OF KIDNEY NEEDLE BIOPSY IN NEPHROTIC SYNDROME

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There have been significant advances in our knowledge of nephrotic syndrome in the past few decades. Nevertheless there are many challenging aspects concerning its cause, the site of injury the physio pathological correlations and the place it occupies in the natural history of glomerulonephritis. Numerous attempts have been made to gain a better insight of the problem These include experimental, physiological histological and immunological studies. One of the most important milestone along the path of progress in the acquisition of more accurate knowledge regarding the nephrotic syndrome was the introduction of percutaneous kidney needle biopsy placed on a sound footing by Iversen and Brun in 1951

Nephrotic syndrome has been defined as a cl n cal ent ty characterized by massive proteinuria with associated hypoproteinaemia, hypoalbuminemia and hyperlipoemia with or without oedema, haematuria hypertension and azotaemia (Derow) It must be realized that the nephrotic syndrome is but a syndrome with many diverse underlying pathological causes, renal as well as systemic, and that n no way should this be regarded as final diagnosis. Even with the exist ng clinical biochemical and immunological studies it is not always possible to arrive at a correct etiological diagnosis clinical diagnosis in a number of such cases remains obscure and the exact pathology unknown due to paucity of autopsy material in our country Th introduction of percutaneous kidney needle biopsy has opened wide perspectives for deta led histologic studies of the lesions. Renal biopsy undertaken at various stages of the disease has made it possible to arrive at a correct etiological diagnosis and to study the exact pathological changes taking place in the kidney

Twenty nine patients, having a clinical picture of nephrotic syndrome were stud ed thoroughly as regards history of illness physical findings and laboratory investigations Renal needle biopsy was performed in all the cases by a modified Vim-Silvermann's needle w th the patient n sitting position. In five cases successful second biopsy was performed Histological changes as revealed by biopsy material were studied in detail

Kidney biopsy was found to be a safe and reliable procedure with only slight discomfort to the patient. No mortality was observed in this series of cases and morbidity was sligl t No major complications were encountered except for the occurrence of an ria in a case. Microscopic haematuria was observed quite frequently wh le frank haematuri occurred in one case only Ou t of 54

biopsy attempts made on 37 patients with nephrotic syndrome adequate renal tissue was obtained in 77.7 % cases. Most biopsies consisted of a cylinder of tissue measuring 8-15 m.m. in length the longest being 26 m.m. in length. The number of glomeruli varied from 0-30 the majority contained 5-20 glomeruli. The tissues were considered to be adequate when five or more glomeruli and the adjacent tubules were present in the section. At times the glomerular changes were so characteristic that even a small number of glomeruli were considered sufficient for an accurate histologic diagnosis.

Biopsy study in two cases with normal gross and discrete renal functions showed large capillary tufts devoid of blood and the cells of proximal convoluted tubule gave an appearance of "cloudy swelling". The presence of proteinaceous material in Bowman's space and tubular lumen confirms the concept that protein is filtered by th glomeruli and reabsorbed by the tubules.

In four patients with subacute glomerulo-nephritis (hydraemic type) parietal epithelium of Bowman's capsule was partly composed of cuboidal or columnar cells instead of flattened cells. The ages of these patients varied from 12 to 25 years. All had the characteristic features of nephrotic syndrome, viz. massive albuminuria, hypoproteinaemia oedema and hypercholesterolaemia. The exact significance of this type of epithelium is not known. It has been suggested that it may be related to functional activity of nephron rather than with pathological changes.

Out of 29 cases of nephrotic syndrome in which successful kidney biopsy was performed 22 were males. The known duration of symptoms at the time of admission varied from 2 weeks to two and half years. The ages of these patients varied from 7 to 60 years the maximum incidence being in the third decade. The histological examination of biopsy material has established that nephrotic syndrome is a clinical entity which may be expression of widely different pathological findings and clinical course. Out of 29 cases studied 9 were due to renal amyloidosis 1 due to lupus erythematosus and the rest were due to glomerulonephritis in various stages of its evolution.

Amyloidosis is an important cause of th nephrotic syndrome. In this series 31 % patients had renal amyloidosis. Clinically it could be diagnosed only in one case where congo red test was positive. In rest of the eight cases it was only after study of histopathological sections of renal tissue obtained on biopsy that a final diagnosis could be made. Though the average age of patients was 34.3 years the disease was quite commonly encountered in third decade as well pointing thereby that amyloidosis is not infrequent in young age. Primary amyloidosis was encountered in 66% of cases. It was observed that in no case amyloid was found in liver biopsies though renal biopsies showed plenty of amyloid material. Depending upon the degree of involvement of glomeruli renal amyloidosis could be graded into three stages histologically

viz., early amyloidosis, moderate amyloidosis and advanced amyloidosis. In cases of early amyloidosis only a few glomerular capillaries showed amyloid deposition in moderate amyloidosis majority of the glomerular capillaries were involved and there was mild tubular atrophy while in advanced renal amyloidosis massive deposits of amyloid material in the glomerular tuft with marked tubular atrophy were noted Patchy pyelonephritis was a common feature in the majority of cases of advanced amyloidosis

One 30 years old female patient with all the features of nephrotic syndrome, had characteristic squamopapular hyperpigmented patches over head, face, chest hand and back. L. E. cell phenomena was present. Histological examination revealed a picture of subacute lupus nephritis.

The clinical picture of oedema albuminuria hypoproteinaemia and hypercholesterolaemia was observed in different stages of glomerulonephritis in the remaining ninteen cases Eight patients had subacute hydraemic glomerulonephritis, one had acute nephritis four had subacute azotaemic nephritis, four had subacute nephritis with early changes of chronic glomerulonephritis while two cases had chronic glomerulonephritis.

From a study of cases of glomerulonephritis responsible for nephrotic syndrome, it is evident that the underlying pathological process in some cases was rapid while in others of slow progress. A complete picture of the gradual evolution of the disease process can be visualised. The case of acute nephritis showed typical proliferative features of the disease These are quite prominent in cases of subacute azotaemic glomerulonephritis. Enlarged glomeruli with increased cellularity due to proliferation of endothelial and epithelial cells, capsular adhesions, a few shrunken and hyalinized glomeruli and the presence of epithelial crescents are evident In advanced cases characteristic picture of chronic glomerulonephritis with many hyalinized glomeruli, islands of hyper-trophied and dilated tubules and of atrophied tubules and sclerotic vessels are seen. In some of the fibrous and cellular crescents pseudotubule formation is observed.

In other group of cases the glomerular changes are mild but tubular degeneration is marked. In very early stages glomeruli show dilated fixed patent, empty glomerular vessels Slight thickening of basement membrane of the glomerular tuft can be made out in these cases. Capsular spaces and tubular lumina contain abundant proteinaceous material. Severe tubular degeneration with considerable fat deposition in the tubular epithelium and well marked oedema of interstitial tissue is seen. In more advanced cases, there is definite thickening of basement membrane of glomerular capillary tufts. Stray capsular adhesions and increase in the lobulation of glomerula tuft can be seen Some of the glomeruli show early hyalinisation. Later on a picture of chronic glomerulonephritis may be obtained Other cases are seen which present a picture of subacute nephritis but without crescents. These cases pro

represent transition between hydraemic type and azotaemic type of glomerulonephritis as suggested by Dible and Davies.

The clinical features in some cases did not correspond with the histological picture Patients having haematuria or azotaemia that is an azotaemic picture showed evidence of hydraemic nephritis histologically with minimal glomerular lesions Probably all these manifestations are part of the natural evolution of the disease. The designations of hydraemic and azotaemic types of nephritis are arbitrary ones and donot indicate fundamental etiologic or morphologic differences but rather the degree to which the capillary lumen are encroached upon, the severity of the changes in the basement membranes and other morphologic phenomena.

Renal biopsy is very useful in giving a prognosis in patients ill with nephrotic syndrome Usually patients with hypertension and considerable azotaemia portended a gloomy outlook The diagnosis of renal amyloidosis is extremely useful from the point of view of prognosis because the expected life span in renal amyloidosis is much shorter than that in glomerulonephritis. In glomerulonephritis the extent and severity of glomerular involvement is an important factor The prognosis is better in cases with least involvement of glomeruli as in early stages of subacute glomerulonephritis (hydraemic type) The question which lesions may revert to normal with consequent recovery from the nephrotic syndrome is of fundamental importance It may well be that the milder forms of glomerular involvement in the absence of systemic disease are capable of regression to normal whereas the more severe forms of glomerular involvement with or without coexistent systemic disease, tend to progress with eventual obliteration of the involved glomeruli

In general albuminuria is inversely proportional to the degree of glomerular involvement. In cases with subacute hydraemic glomerulonephritis and in early amyloidosis where the glomerular lesion is least massive albuminuria is present while in chronic glomerulonephritis or advanced amyloidosis with marked involvement of glomeruli it is slight. No definite relationship can be established between the degree of oedema and renal damage Azotaemia may be present with or without severe involvement of glomeruli in cases of glomerulonephritis while in renal amyloidosis it may not be present even with extensive glomerular damage.

For a rational treatment of nephrotic syndrome with steroid therapy it is essential to understand the underlying pathology No means are available for predicting which patients would respond to ACTH or cortisone Patients with gross and microscopic haematuria with moderate degree of renal insufficiency and hypertension have been reported to have responded to therapy Six out of seven patients with subacute glomerulonephritis who responded favourably to prednisolone therapy were under 14 years of age The response in older age group was not favourable In general, the response to predni

solone therapy was much better in cases that showed severe oedema massive proteinuria hypoalbuminemia and no or mild azotaemia with normal blood pressure. The histological picture in these cases varied from those of hydraemic nephritis to those of azotaemic nephritis with changes of chronic glomerulonephritis. It was difficult to predict from the histological appearances the nature of response to steroid therapy

The histological appearance in renal biopsy material after steroid therapy did not show marked change. In one case of subacute hydraemic nephritis, post therapy biopsy showed early changes of chronic glomerulonephritis, though clinically the patient showed complete recovery It suggests therefore that though steroid therapy removes oedema and reduces albuminuria the glomerular lesions persist or may advance. It conceals rather than eliminates the underlying pathological process.

As a research tool for the investigation of the pathological development of various forms of nephritis needle biopsy holds great possibilities. Quite apart from its obvious value in following the progress of various lesions needle biopsy is of great diagnostic importance and of crucial value in determining the correct diagnosis and hence management of particular case.

ANALYTIC STUDY OF THE HABITATS IN THE RIVER AVON AT GREAT DURNFORD

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A. Physico-Chemical Conditions.

Introduction

The river Avon is a typical chalk river traversing the chalk regions of Southern England. It has its origin some 20 miles north of Salisbury at an altitude of about 350 ft. in the Greensand plateau of the Vale of Pewsey (Ordnance sheet No. 167) where it has two principal tributaries—one coming from the north-east beyond the area, and the other from the north-west. These tributaries unite between Rushall and Upavon. As a result of its origin from the Greensand (Jukes-Browne 1905) the river is acidic in its upper reaches, but later as it passes over chalk country and is fed at places by springs from the chalky bed, it changes from acidic to alkaline. In most of its extent the Avon flows over broad gravel and alluvial terraces. At intervals it is joined by other rivers, not all of which originat from the same geological stratum. Above Salisbury it is joined by the rivers Wylye, N dder and Bourne. Tabl 1., shows the variations in pH of the river Avon from place to place.

Table 1

pH of river Avon at different places (15th September 1952)

Upavon	½ mile below Upavon Bridge	1½ mile below Upavon Bridge	Enford Bridge	Coomber Bridge	Haxton Bridge	Netheravon	½ mile below Nether Bridge	Bulford Power station	Great Durnford
7 15	7 85	7 8	7 9	8.1	8 1	7 4	7 4	7 6	8.0

In summers the water level in the river drops, the depth varies from 3 to 6 feet, at various places, and weeds grow widely During winters the water level rises, the depth varies from 4 to 8 feet. The weeds die out or get submerged under water or are cut down

Determinations of pH calcium, chlorides and percentage oxygen saturation were made at Great Durnford during months of July and September 1953 and in January 1954 On each occasion the determinations of oxygen and pH were made every 3 hours over a period of 24 hours

Weekly records of maximum and minimum temperatures of the river water were also kept from August 1953 to March 1954

OXYGEN

The percentage saturation of oxygen was estimated at the river bank by means of a modified Winkler s method using a syrine pipette (Whitney 1938) Table 2. and Fig 1 contain the results.

Table 2

Oxygen percentage saturation and pH of river Avon waters at Great Durnford

Time	Oxygen parts per 100 cc.			Oxygen saturation			pH		
	July 10th 11th.	Sept. 9th. 10th.	Jan 12th 13th	July	Sept.	Jan.	July	Sept.	Jan.
9 hrs	5 875	4 671	6 573	83	65	77 0	8.2	8.2	8.0
12 hrs.	7 360	7 219	9.342	105	102	112 0	8.2	8.2	8.0
15 hrs.	8.204	8 352	7 927	120	120	93.0	8.3	8.3	8.0
18 hrs	9 131	6 794	6 794	130	97	81 0	8.3	8.2	7 9
21 hrs.	6 158	5 662	5 237	89	78	62 0	8.2	8.2	7 9
24 hrs.	4 742	4.247	6.511	67	57	77.5	8.0	8.1	7 9
3 hrs	5.521	4 671	6 370	77	62	78 0	8 0	8.0	8.0
6 hrs.	5.095	3.022	6.791	73	52	77.5	8 0	8.0	7 9
9 hrs.	5.803	4 388	6 087	83	60	74 0	8 1	8.1	8.0

The general character of the diurnal curves in the three months is the same i. e., the oxygen content rises shortly after sunrise to a maximum at about mid-day and then it falls to a minimum in the hours of darkness. But there are differences in the three months as far as the quantity of oxygen is concerned. In July the maximum percentage saturation is 130 per cent at 18.00 hours, while the minimum is 67 per cent at 24.00 hours. In September the maximum percentage saturation is 170 per cent at 15.00 hours and the minimum 52 per cent at 6.00 hours. During winter i. e., January the maximum percentage saturation is 112 per cent at 21.00 hours. As is already known the variation in oxygen saturation is due to photosynthesis by plants and the temperature changes.

Hydrogen Ion Concentration

It has been shown in Table 1 that the pH of the river varies from place to place. The pH in the river Avon depends mainly upon the amount of $CaCO_3$, $Ca(HCO_3)_2$ and CO_2 dissolved in water. The determination of pH was made by the addition of phenol red and thymol blue to the river water and comparison with B. D. H. standard buffer solution. No greater accuracy than the nearest 0.2 was obtained. Table 2 shows that at Great Durnford the pH is almost constant throughout the year with only slight diurnal and seasonal variations.

Calcium

The amount of calcium was determined in July and September 1953 and January 1954. It was precipitated as oxalate, the precipitate was dissolved in dilute sulphuric acid and titrated against standard potassium permenganate solution. The results show that the amount of calcium was minimal in July (Table 3) and maximal in January. Perhaps the greater rainfall in winter added more calcium to the river from the surrounding hills.

Table 3

Amount of calcium and chloride in grams per litre

Calcium			Chloride		
July	September	January	July	September	January
0.045	0.093	0.114	0.021	0.019	0.021

Chloride

The chloride content was estimated in July and September 1953 and January 1954. The river water was titrated against standard 0.1 N silver nitrate solution. Table 3 shows that the amount of chloride present in the river water was nearly constant.

Temperature

Weekly records of temperature were kept from August 1953 to March 1954 Table 4 contains the average minimum and maximum temperatures for each month calculated from the weekly figures.

Table 4

Average maximum, minimum and mean monthly temperatures (°C) of the River Area.

	1953					1954		
	Aug	Sept.	Oct.	Nov	Dec.	Jan.	Feb.	Mar
Maximum	18 3	17 2	11 6	10 5	10 5	5 5	1 6	5 5
Minimum	14 4	10 0	7 7	6 1	8 3	2 7	0 6	3 3
Mean	16 4	13 6	9 7	8 3	9 4	4 1	0 5	4 4

In 1954 February was unusually cold Generally however in both January and February the temperature is about 4°C. Probably July and August are the warmest months when the temperature may exceed 18 5 C.

B The Invertebrate Fauna.

Material and Methods

A variety of methods have been used by different workers for making comparable faunal collections from a stream. Percival and Whitehead (1926) used a specially designed shovel net Whitehead (1935) later used a box for making collections from the weeds. Macan and Worthington (1951) devised a hand operated grab, and Allen (1940) a scoop for the same purpose. Beak (1937) used a grab which was similar in principle to the Petersen type and Allen (1952) worked with a hand operated grab

The Petersen s grab and the apparatus designed by Allen are not very successful on a stony river bed. They have jaws liable to jam when the grabs close. Many animals might be lost by the scoop devised by Allen (1940)

In the present investigation the Surber stream-bottom sampler with slight modifications (Fig ?) was used for sampling in firm or gravel beds. The original-Surber s sampler (1937) consisted of a one foot cube frame covered with canvas on two opposite sides. A nylon bag was attached to the third side of the frame while the side opposite to the bag was kept uncovered In the present work this last side was also covered with a nylon mesh. A two-inch wide strip of sponge rubber was stuck to the bottom of the frame These two alterations were necessary in view of the following facts :

(i) If Surber's net were to be used without a front window of nylon mesh, organisms would enter the net here from outside the area enclosed by the frame of the sampler. The mesh allows a flow of water but not solid particles and organisms into the enclosed area. When the bed of the river is stirred with a stick, materials and organisms dislodged are carried into the net by the water current entering through the window.

(ii) Owing to irregularities of the substratum when the original Surber's sampler is put on a stony bed, some space is generally left between the frame and the river bed. The sponge rubber affixed to the frame by the author serves to seal up such gaps.

The modified Surber's sampler was kept stationary in the water with a handle attached to the frame. This type of net can be used only in a fast flowing river with a sandy or gravelly bottom.

For obtaining samples from weed-infested habitats as well as from the muddy bottom of the river, a hand-net was used with a mesh-work of 48.5 per inch. The net was held under the weeds which were cut with a pair of scissors and collected in the net. When collection from a muddy bottom was made, the hand-net was pushed into the mud and dragged up the stream for a distance of about one foot. Most of the mud was washed away through the net and the remainder was brought to the laboratory.

The collections made at Great Durnford were from three different habitats from amongst the vegetation, the muddy bed and the gravel substratum of the river. In January, March, May, July, August and November 1963 samples were collected once a month, on each occasion three times from each of the three habitats, the results presented being the mean of these three. None of the above habitats was completely devoid of vegetation.

Every sample was washed through a series of sieves with the aid of a jet. The contents of each sieve were emptied into a white enamelled dish, counted and preserved in 5% formalin for precise identification. Some of the organisms suffered damage to some extent from sieving; this was especially the case with *Gammarus pulex* and some Ephemeroptera nymphs which were abundant in many of the samples. Not much difficulty, however, was experienced in counting the intact heads.

Animals thus collected, are listed in Table 5 in terms of their relative abundance which is indicated as follows:

R——Rare. This denotes animals rarely present in the collection, their number being less than 5 per sample.

F——Few *i.e.*, 5 to 10 per sample.

C——Common. The animals numbered between 10 to 50 per sample.

N——Numerous. These were of very common occurrence, ranging between 50 to 100 per sample.

A——Abundant., *i.e.*, those which occurred in hundreds per sample.

The three different habitats, *i e* gravel mud and vegetation are represented by G M' and 'V' respectively

RESULTS

Table. 5 shows the organisms found at Great Durnford and their relative abundance at different times of the year Of these, the following may be specially mentioned

MOLLUSCA The most common species is *Hydrobia jenkinsi* It is abundant in the vegetation and shows little seasonal variation in its density of population *Theodoxus fluviatilis* occurs throughout the year

CRUSTACEA *Gammarus pulex* occurs quite commonly and at times is abundant. It generally inhabits the vegetation and gravel The presence of a certain amount of vegetation in the muddy region may account for its presence in that environment. There is a decrease in the number of *Gammarus pulex* in winter months (Berg 1948) A similar variation is seen in case of *Asellus aquaticus* which is common in May

EPHEMEROPTERA Nymphs of *Ephemera danica*, *Ephemerella ignita*, *Baetis* sp., and *Caenis* sp are of common occurrence Since *E danica* nymphs generally take two years to develop they may be found almost throughout the year They are numerous during winter but less common in other months. They generally inhabit the gravel *E ignita* nymphs occur from May to August. During the rest of the year this species is said to exist in the egg-state (Macan private communication 1953) Nymphs are numerous in August and are generally found amongst vegetation. *Baetis* sp nymphs are commoner in winter than in summer *Caenis* sp., nymphs are found in every collection but they also show a seasonal variation and are common in winter

DIPTERA LARVAE AND PUPAE Chironomidae larvae form an important item of the river Fauna. They generally inhabit all the three habitats. No marked seasonal changes seem to occur in the population of Chironomid larvae, since different species emerge at different times of the year (Humphries and Frost, 1937)

Simulium sp., larvae are numerous in summer while they are common in other seasons They generally inhabit the vegetation and gravel.

Dipteran pupae (mostly chironomids) are common in May and August.

Table 5

Fauna of river Avon in different months in the three selected habitats

	January			March			May			July			August			Nov		
	G	M	V	G	M	V	G	M	V	G	M	V	G	M	V	G	M	V
Theodoxus fluviatilis	R	R	R	C	C	—	R	R	R	—	R	—	R	R	—	C	R	—
Ancylastrum fluviatile	—	—	R	—	—	R	R	R	—	—	—	F	R	—	—	R	—	—
Ancylus lacustris	—	R	R	R	—	R	—	—	—	—	—	F	—	—	—	R	R	R
Bithynia tentaculata	—	R	R	—	—	—	—	—	—	—	—	R	—	—	—	R	—	C
Physa fontinalis	—	—	R	—	—	R	R	—	—	—	—	—	—	—	—	—	—	—
Valvata piscinalis	—	—	F	—	—	—	—	—	F	—	R	—	F	—	F	—	R	F
Limnea peregra	—	—	R	—	—	R	—	—	—	—	—	—	—	—	—	—	R	F
Planorbis sp	—	R	R	—	—	—	—	—	R	—	—	—	R	—	—	—	R	F
Sphaerium	R	F	C	R	—	—	C	—	—	—	—	R	F	—	—	C	F	F
Hydrobia Jenkinsi	—	C	A	F	—	A	F	C	A	F	C	A	C	—	A	—	A	A
Gammarus pulex	C	F	C	N	C	N	N	F	A	N	F	N	N	F	C	N	R	C
Asellus aquaticus	—	—	C	—	—	F	F	—	C	F	—	F	R	R	C	R	F	C
Sialis sp. larvae	R	R	R	—	—	—	—	—	F	—	—	—	—	—	—	R	C	R
Corixidae nymphs	—	—	F	—	—	—	—	—	F	—	—	—	—	—	F	—	F	—
Ephemera danica nymphs	N	F	R	C	—	—	F	—	—	C	—	—	C	F	—	N	R	—
Ephemerella ignata nymphs	—	—	—	—	—	—	—	—	C	—	R	C	—	C	N	—	—	—
Baetis sp. nymphs	F	—	C	—	—	N	—	—	C	R	—	F	—	—	C	F	—	C
Caenis sp. nymphs	F	C	—	—	C	—	F	F	F	—	—	R	F	F	R	C	R	—
Plecoptera nymphs	—	—	F	—	—	—	—	—	F	—	—	—	—	—	F	—	—	—
Chironomidae larvae	C	C	A	N	R	C	F	C	A	C	C	A	A	C	N	N	N	F
Simulium sp. larvae	C	—	F	C	R	C	—	—	—	C	—	N	C	—	N	C	—	—
Other diptera larvae	—	F	F	R	—	R	—	—	F	—	—	R	—	—	F	R	R	F
Diptera pupae	R	—	—	—	—	—	F	—	C	F	—	C	F	F	C	—	—	F
Helmidae larvae	F	R	F	F	F	—	—	—	—	—	—	—	F	—	—	N	—	F
Coleoptera adult	F	—	—	F	F	—	C	C	—	F	—	R	R	F	F	F	—	R
Trichoptera larvae	C	C	A	C	R	A	A	R	N	C	R	A	C	—	C	A	R	C
Trichoptera pupae	C	C	C	—	—	—	—	—	C	—	—	—	—	—	—	—	—	—
Oligochaeta	C	N	—	N	A	—	C	C	C	F	C	—	F	C	—	—	—	—
Hirudinea	—	R	R	—	R	—	R	—	—	F	—	—	—	F	—	—	—	—
Hydracarina	R	R	R	R	R	R	F	F	F	R	—	R	R	—	F	C	R	F

G	Gravel.	A	Abundant(100)
M	Mud.	N	Numerous(50-100)
V	Vegetation	C	Common (10- 50)
		F	Few (5- 10)
		R	Rare (1 5)

TRICHOPTERA Trichopteran larvae form another important item in the Fauna. They are numerous in practically all the months but show a decrease in August when they are only Common. Trichopteran pupae are common in January. Their favourite habitate is gravel or vegetation according to the species.

COLEOPTERA Adults are common in May and 'Few' in other months. Helmidae larvae are numerous only in November

CONCLUSIONS

The River Avon although a typical chalk river is especially important as it shows two distinct zones as far as pH is concerned. It arises some 20 miles north of Salisbury and traverses the chalk-regions of southern England. Near its origin it flows over acidic greensand but later as it passes over chalky country its water changes from acidic to alkaline. In the vicinity of Great Durnford the pH ranges from 7.4 to 8.4 in various seasons and localities. Thus it is virtually constant throughout the year although it shows slight diurnal and seasonal changes.

A determination of calcium content of the river water shows that calcium was minimal in July and maximal in January. This may perhaps be due to the fact that greater rainfall in winter brings with it more of the leached-down calcium from the surrounding area, thereby enriching the river water in January.

The amount of chloride present in the river-water remained almost constant in the three months selected for investigation—July, September and January.

The temperature was lowest in January and February—the mean average being 4°C. July and August are the warmest months when the average temperature may exceed 18.5°C.

Most of the river fauna occurs in the gravel and vegetation habitats. The major proportion of it as shown by the present sampling consists of *Hydrobia jenkinsi*, *Gammarus pulex*, Ephemeropteran nymphs, Chironomid larvae, *Simulium* sp. larvae, Trichopteran larvae and Oligochaeta.

No marked seasonal differences were observed in the total numbers of the animal population in the river. This was so because, when one type of organism diminishes in number, the other forms increase.

BIBLIOGRAPHY

1. ALLEN, K. R. H. (1952): A hand-operated quantitative grab for sampling river beds. *J. Anim. Ecol.*, 21 pp. 159-160.
2. ALLEN, K. R. (1941) Studies on the biology of the early stages of the salmon. II. Feeding habits. *J. Anim. Ecol.*, 10, pp. 47-76.
3. BEAK, T. W. (1936-1937) Methods of making and sorting collections for an ecological study of stream. *Ann. Bio. Riv. Assn. Rep.* No.5 pp. 42-47.
4. BERG, K. (1948) Biological studies on the River Susaa. Folia Limnologica Scandinavica No. 4.
5. HUMPHRIES, C. F. & FROST, W. E. (1937) River Liffey Survey. The Chironomid fauna of the submerged mosses. Proc. R. Irish Acad., 43 Sec. B, (11).
6. JUKES-BROWNE, A. J. (1905) The geology of the Country South and East of Devizes. Mem. geol. Surv. U. K. England and Wales. Explanation of sheet 282.
7. MACAN, T. T. & WORTHINGTON, E. B. (19 1) Life in lakes and rivers. Lond.

8. Percival, E. & Whitehead, H. (1926): Observations on the biology of the mayfly *Ephemera danica* Mull. *Proc. Leeds Phil. Soc.* (*Science Sec.*) 1

9. Surber, E. W. (1937): Rainbow trout and bottom fauna production in one mile of stream. *Trans. Amer. Fish. Soc.* 66.

10. Whitehead, H. (1935): An ecological study of the invertebrate fauna of a chalk stream near Great Driffield York. *J. Anim. Ecol.* 4, pp. 58-78

11. Whitney, R. J. (1938): A syringe pipette method for the determination of oxygen in the field. *J. exp. Biol.* 15, (4) pp. 564-570

Fig. 1. Diurnal changes of oxygen percentage saturation, temperature, and pH
Shaded portion represents hours of darkness.

Oxygen percentage saturation

Temperature.

pH value.

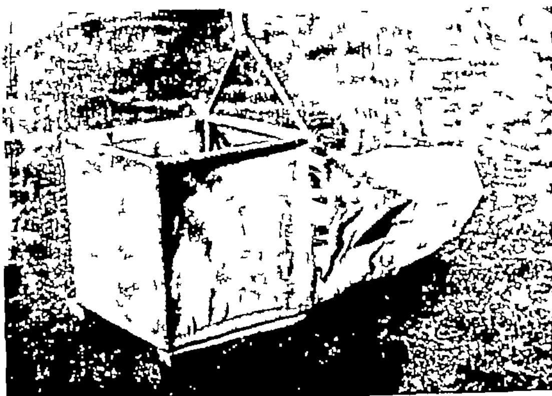


Fig. 2. Modified Surber's stream bottom sampler. The gauge of the net used was 48·5 M. P. I.

MORPHOLOGY OF *TOR PUTITORA* (HAMILTON)*

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The Thesis provides a detailed general account of the morphology of the fish *Tor putitora*, with a short resume of relevant literature Bibliography for all the systems is given at the end

Hamilton was the first Biologist who described the species under the name *Cyprinus (Cyprinus) putitora*. McClelland recorded it as *Barbus putitora*. Day included the Putitor mahaseer in his Monograph of Indian Cyprinidae and added a short note on its breeding habits; Thomas made certain observations about the fish in his book, *Rod in India* Hora and Mukerji recognised it as *Barbus putitora*. Hora finally revised again its systematic position and recognizing *Tor* as a subgenus recorded it under the name *Barbus (Tor) putitora*. Misra described it under the name *Tor putitora*.

Tor putitora is distributed along the Himalayas in mountainous or rocky streams. It has been reported from Burma Ceylon Baluchistan Waziristan and Afghanistan and also from Mahanadi river (South India)

The causes assigned for the migration are breeding, temperature variations, safety of the spawn and search for food. The migration limits are well-known and the fish is not found in rivers rising below 1,000 feet above the sea level while the upper limit of the migration is about 6,000 feet for India. The best breeding grounds lie upstreams the fish migrates to the higher reaches from its summer habitat for breeding and returns with the receding floods.

In structure and appearance Mahaseer conforms more or less to a typical carp The body is distinguishable into the head trunk and tail regions. The head is dorsoventrally compressed. The mouth is small and its gape is not wide. The lips are thick and fleshy Eyes are large and round. Nostrils lie near the eyes and there are two pairs of barbels The trunk region is provided with paired and median fins. The tail fin is situated at the posterior end. The tail is homocercal. Large cycloid scales cover the body The lateral line runs along each flank of the body An adult fish is reddish green on the dorsal surface, while its ventral surface is provided with light orange fading into silvery white at the mid-ventral line

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The skin of *Tor putitora* is composed of two layers, an outer epidermis and an inner dermis. The epidermis consists of many layers of epithelial cells. The outermost layer of cells is formed by two or three layers of flat cells which are elongated and run parallel with the surface The mucous cells are few in number but the presence of many clavate cells is peculiar Clusters of sensory cells are located in the outer layers of the epithelial cells. The cells of *stratum malpighi* possess a dense cytoplasm and thick oval nuclei

The dermis consists of elastic fibres, reticular connective tissue and collagen fibres, along with blood vessels and nerve endings.

The entire body except for the head and fins is covered with large cycloid scales. The scales are thin flexible regularly arranged like the tiles of a house Each scale has a front end inserted deep into a pouch in the dermal layer of the skin A scale examined with a hand-lens or under a low power of the microscope reveals that it is composed of well defined radii and circuli arranged around a focus The scales of *Tor putitora* are the largest of all the fresh-water fishes of India

The vertebral column is composed of forty to forty three vertebrae. In a typical trunk vertebra various components of a vertebra along with parapophyses can be distinguished The first four vertebrae are modified in relation to the Weberian apparatus. The four elements of the Weberian apparatus are well developed.

In a typical caudal vertebra, the haemal arch is distinct and the neural spine is directed obliquely backwards. The last three caudal vertebrae are modified to support the caudal fin In the first two caudals the *neural* and haemal spines fuse with the corresponding radials and are flattened laterally at their distal ends. The centrum of the last caudal vertebra is flattened sideways and gives rise to several rod-like processes on the dorsal side The *urostyle* is formed by the fusion of a number of vertebrae in this region. The neural and haemal spines fuse with the corresponding fin radials and form epurals and hypurals respectively

The skull consists of the cranium the sense capsules and the visceral arches, which form the jaws, the suspensorium and the hypobranchial skeleton for the support of the gills.

The occipital region constitutes the posterior part of the skull. It is composed of four bones the supra-occipital, the two exoccipitals and the basi-occipital The auditory capsule consists of the prootic the epiotic the sphenotic and the pterotic bones. The opisthotic is absent The orbito-temporal region is differentiated into the orbital and temporal regions The former includes bones of the orbits and the latter is sub-divided into th frontal and parietal regions The ossicles which surround the eye are large and together form a compact orbital ring The frontals are remarkably well developed and

compensate to a great extent for the poor development of other bones. The parietals are prominent and complete the roof of the cranium on the postero-dorsal surface.

The ethmoidal region is situated at a lower level than the frontal region and constitutes the anterior most part of the skull. It includes the mesethmoid, the ecto-ethmoid the nasals, the vomer and the lacrymals. The vomer is a prominent, median bone which develops antero-dorsally two knob-like prominences, vomerine processes, a special structure not generally met with in teleosteans.

In the visceral skeleton the mandibular arch consists of the palato-pterygo-quadrate and the Meckel's cartilage bars. The primitive upper jaw is ossified into three replacing bones, the palatine, the pterygoid and the quadrate, but the functional upper jaw is formed by independently developed paired dermal bones the premaxillae and the maxillae. Three bones a dentary an articular and an angular together form the lower jaw of the adult fish.

The hyoid arch consists of two distinct portions the dorsal including the hyomandibular and the symplectic, and the ventral forming the hyoid cornu.

The main elements of the hypo-branchial skeleton are the epihyal the cerato-hyal and a double hypo-hyal. These are attached to a median basi hyal. Connected with the hyoid arch are four investing bones which support the operculum and are developed on either side of the posterior border of the skull.

The first four branchial arches support the gills and the pharyngeal wall, and the last is reduced to a single bone, the inferior pharyngeal bone.

The dorsal ribs extend outwards in the horizontal septum at its inter section with the transverse myosepta. The ventral or the pleural ribs extend downwards where the myosepta join the coelomic wall. Besides these pleural ribs, there is a second series of small and needle like bones, the epi-pleurals.

The median fins include the dorsal the ventral and the caudal fins. Their skeletal elements are made up of somactidia and the dermotrichia. The dorsal fin comprises of twelve or thirteen fin-rays which are seated on ten radials. The ventral fin consists of seven or eight fin-rays which are seated on six radials. The caudal fin includes the urostyle two epurals and a free radial on its dorsal surface, while nine hypurals are located on its ventral surface

Seven unpaired pieces—the neural plates are present in between the neural spines of the vertebrae from fifth to twelfth. These have not been reported so far in other teleosteans.

In the digestive system the alimentary canal is of the normal teleostean type. In the buccal cavity the maxillary oral valve is present. The mucous membrane lining the buccal cavity has low longitudinal folds. In the pharyn-

geal region the horny pad and the pharyngeal teeth are well marked on the fifth pair of gill arches The gill rakers are large and prominent The oesophagus is followed by the intestinal bulb which presents well developed mucous folds seen with the naked eye. The long intestine is of varying diameter The glands associated with the alimentary canal are a bilobed liver and the pancreas which is diffused and scattered.

Judged by the length of the intestine and the diversity of food elements consumed, the Mahaseer is regarded as an omnivorous fish Feeding habits of the fish are characteristic. The lips which are fleshy and well developed help to take in mud and other food materials from the bottom The barbels are used for exploring food at the bottom and on the rocks. The pharyngeal teeth are strong and conical and appear to be very efficient for mastication of the diverse food elements.

In the respiratory system of the fish there are four pairs of the gills borne on the first four branchial arches in the branchial chamber The gills are of filiform type. The fifth arch which is modified into an inferior pharyngeal bone, bears no gill lamellae.

The interbranchial septum is reduced and confined to the base of the gill arch The pharyngeal wall is *perforated by* gill slits which *open together* in the branchial chamber The opercular bones form the outer wall of the branchial chamber Along the posterior border of the operculum lies the branchiostegal membrane

The deoxygenated blood is brought to the gills by the afferent branchial vessels while the aerated blood is carried away by the efferent branchial vessels.

Respiratory mechanism comprises of inspiratory and expiratory phases. Water enters the mouth cavity then passes to the branchial chambers through the pharynx and finally passes out through the gill slits The exchange of respiratory gases takes place by diffusion over the surface of the gill filaments.

Most hill-stream fishes appear capable of suspending their respiratory movements for a shorter or longer period This periodic suspension of the respiratory movement is rendered possible by the fact that the water is retained in the branchial chambers when the gill openings are reduced or closed

The swim bladder is a large shining structure situated on the dorsal side of the abdominal cavity below the vertebral column. It is divided into a small anterior and a larger posterior portion. The two portions are in communication with each other through an opening

The long thin pneumatic duct arises from the front end of the posterior portion. It opens into the anterior part of the oesophagus. Its histological

structure is also described. The swim bladder develops an association with the internal ear through the Weberian ossicles. The heart of *Tor putitora* resembles that of a typical Teleost. In the arterial system, the first and second afferent branchial arteries arise directly from the ventral aorta, while the third and fourth afferent branchial vessels arise together through a common opening The first and second efferent branchial arteries enter the circulus cephalicus separately on each side. The common carotid artery is an anterior prolongation of the radix aorta for the supply of the head region The other body parts are supplied by branches of the dorsal aorta.

The veins include the anterior cardinal system which is formed by the orbitonasal, maxillary mandibular and opercular veins The inferior jugular collects the blood from the ventral surface of the head The posterior veins collect the blood from the posterior part of the body Renal portal system is not well developed. The posterior cardinals are not symmetrical the left one being greatly reduced. The lateral system of veins collects the blood from the muscles and lateral sides of the body The hepatic portal system includes the accessory caudal vein and the veins from the alimentary tract

The excretory organs of the fish include the kidneys which are peculiar in shape and structure. Each kidney is divided into the head mesial and hind kidneys. It fuses with its fellow of the opposite side completely in the mesial portion and it is difficult to separate the nephrogenous tissue of the two kidneys. The excretory function is not confined only to the posterior regio but extends also in the middle portion. The ureters arise from the mesial portions of the kidneys on the outer margins. Each ureter runs up to the hind end of the cloaca where it joins with its fellow of the opposite side. The median tube dilates to form the urinary bladder which is a thin-walled sac opening to the exterior through an aperture near the anal opening It s an oviparous form and the sexes are separate. Each testis is d vided imperfectly into three lobes. The vasdeferens arises from the posterior end of each testis. It runs up to the cloacal region and after joining with its fellow of the opposite side opens into the urinogenital sinus.

Each ovary is an elongated sac in which projects the ovarian tissue in the form of processes. From the posterior end of each ovary arises an oviduct which runs up to the end of the cloaca nd joins with its fellow of the opposite side. The common oviduct opens independently to the outside The gonads are not connected with the kidneys.

In the brain the two cerebral hemispheres are differentiated and the cerebral region is marked by commissures The olfactory lobes lie in front of the rest of the brain In the diencephalon region, the pineal body is distinct on the dorsal surface. Along the ventral surface of the diencephalon region lie the infundibulum the hypophyses the saccus vasculosus and the lobi inferiores. In the mid brain, the optic lobes are conspicuously well

developed. The hind-brain consists of a cerebellum and medulla oblongata. The cerebellum is large and bent upon itself The medulla is roughly triangular and forms the posterior boundary of the brain. The important lobes of the medulla are the facial and vagal lobes.

The cranial nerves the typical ten pairs are represented. The maxillary (v) and the buccalis (vii) are wrapped by a common covering The ophthalmicus profundus is well developed The glossopharyngeal (ix) and branchialis (x) divide into the pre-and post trematic branches The spinal nerves resemble those of a typical Teleost.

The adaptations of the Mahaseer fish to life in hill-streams being primarily the graceful form of the fish which appears to have been evolved as a special adaptation for withstanding the rapid current in the hill-stream. There is a large caudal peduncle and a distinct flattening of the ventral surface of the body from the pharynx to the anus. The paired fins are comparatively large and have a more ventral insertion on the body than in other carps. The most conspicuous modification is of the lips. The mouth with its hypertrophied lips serves as a secondary organ of adhesion in Mahaseer

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INTER-RELATIONSHIP OF BLOOD HISTAMINE AND HISTAMINE LIKE SUBSTANCES IN EOSINOPHILIASIS

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Allergic nature of the pulmonary eosinophilia is believed by Hanson Prum and Goodman (1944) Vaidya (1945) Joseph (1946) and Crofton Livingstone Oswald and Roberts (1952) Schield *et al* (1951) suggested the major role of histamine in human allergic asthma and Rose (1950) found an increased quantity of histamine in the urine of asthmatics.

Code (1952) Achari and Acharya (1955) and Jha (1957) observed, that blood histamine contents of eosinophilia patients is found to be raised in comparison to normal persons Riley and West (1953) and Valentine *et al* (1950) demonstrated that eosinophilic granulome and periarteritis nodosa patient has no increase of histamine in tumour tissue and blood respectively Bronchial asthma and pulmonary eosinophilia in human beings has a very close resemblence. Katz and Cohan (1941) and Chaudhri (1956) showed the release of histamine from blood cells by specific antigen in allergic patients

There has been no comparable evidence to show that histamine is released in human allergic asthma. Cargue (1936) Parrot (1938) showed definite crease in blood histamine while others *e g* Riener (1937) and Rose (1941) found no consistent change

Hence under these controversial circumstances this work of blood histamine bioassay in cases of pulmonary eosinophilia, where this increase in eosinophil cells could not be associated with any other infection was performed with an intention to find the interrelationship of blood histamine and the degree of eosinophiliasis. As, there is little data available of normal blood histamine level in Indian subjects 25 normal persons were chosen of different age groups with different status in their lives to compare the blood histamine level in eosinophiliasis with blood histamine level of normal persons

Blood histamine was extracted from 25 normal healthy and 34 eosinophilic individuals following the Code s modified method (1957) of Barsoum and Gaddum (1935)

The presence of any other pharmacologically active substance was ruled out by heating with hydrochloric acid (Code 1952) and by getting the same degree of inhibitory response with antihistamanic (Antistine) against equipotent

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dose of blood histamine extract and standard solution of histamine acid phosphate on the tracheal chain obtained from normal guinea pigs The latter was chosen for the final bioassay of histamine instead of the widely used tissue i. e. guinea pig's terminal portion of the ileum because of the fact, that the sensitivity of this portion of ileum varied with the repeated same doses of histamine. The concecutive amplitude of contraction of the portion of ileum went on increasing for 6 or 7 doses and then after either the responsiveness gradually decreased or ceased all of a sudden. Experimental variations in temperature time of relaxation different weights for relaxation different doses of histamine and slight change in tyrode s solution chemistry were done but with no appreciable results.

Guinea pigs uterus was also rejected as big rhythemic contractions were observed inspite of increased relaxation time and cat s eviscerated preparation was also not tried for the reasons as discussed under the chapter of discussion No 4

Hence tracheal chain was the test object for the histamine bioassay In the present series normal histamine blood level range was found to be 0 003 to 0 512 mc. gm/c.c. and mean level of 0 02632 mc gm/c.c. as histamine base.

Blood histamine in 34 eosinophilic patients was found to be increased but had no relationship with the increase in absolute eosinophil count. An effort has been made to discuss the possible relationship between eosinophiliasis and blood histamine.

The blood histamine range was determined as 0 06133 to 0.5326 in 31 eosinophilic patients with a mean value of 0 231 mc. gm/c.c as histamine base The standard deviation was calculated as 0 1256 while the standard error of the mean was 0 0215

OPTICAL ACTIVITY AND CHEMICAL CONSTITUTION
Part I—Early Work

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The effects produced by the Polarization of light have been generally known since 1690, the year which saw the publication of *Treatise on Light* by Huygens (Eng tr by S Thomson, Macmillan & Co., 1912) in which are explained the causes of that which occures in reflection and in refraction of Iceland Crystals The first satisfactory explanation of the nature of polarized light was given by Augustin Fresnel in 1821 in a long memoir read before the Academie des Sciences (*Memoire Sur La Double Refraction* read on Nov 26 1821 Jan. 22 and April 22, 1822) According to him Polarized light is that in which the transverse oscillations take place constantly in one direction and ordinary light is the bringing together and the rapid succession of an infinite number of systems of waves polarized in all directions Mention may next be made of the classical experiments of Biot on rotatory polarization as described in five memoirs (Biot, 1812 1817 1835 1838 *) presented to the French *Academie des Sciences* from 1812 to 1838 The earliest part of the memoirs deal with the behaviour of crystals of gypsum and quartz cut parallel to the axis and thus exhibiting the ordinary phenomenon of double-refraction The fifth part of the memoirs entitled *Experiences Sur les plaques de cristal de roche taillees perpendiculairement a l axe de cristallization* describes experiments which led him to conclude the following

(i) Luminous molecules of different kinds which have passed through the plate of rock crystal, have by the action of this plate turned their axes of polarization into different azimuths.

(ii) The violet molecules turn faster than the blue, the blue faster than the green the green faster than the yellow and so on to the red molecules which will be the slowest of all.

Thus he was able to establish simultaneously the phenomenon of rotatory polarization and rotatory dispersion

In these memoirs Biot dealt with crystals of gypsum, Iceland spar plates of mica, quartz and so on but in 1815 he also studied the rotatory polarization in oils of turpentine, laurel and lemon and in alcoholic solutions of camphor (Biot 1815) These studies led him to the general conclusion that the law of rotation of different simple rays is the same for liquid sugar as rock-crystal and oil of turpentine From this one can infer with

probability that the law is a general one. The law referred to is the law of Inverse Squares, namely $\alpha \propto \frac{1}{\lambda^2}$ where α is the rotation for wavelength λ. It is to Biot (1838) that we owe the definition of specific rotatory power $[\alpha]$ viz.,

$$[\alpha] = \frac{\alpha}{l.\ \delta} \text{ or } \frac{\alpha}{l.\ \epsilon.\ \delta} \quad \text{where}$$

α is the observed rotation in degrees, l is the length of the column in dcm. δ is the density of the liquid and ϵ is the fraction by weight of the optically active compound. In a similar way molecular rotatory power [M] is defined as $[M] = \frac{M}{100} \times [\alpha]$ where M is the molecular weight and $[\alpha]$ is the specific rotation. This is now the universally accepted convention. It may be pointed out that Jaeger uses the C. G. S. system for defining molecular rotatory power and Hudson uses $[M] = M \times [\alpha]$ and hence their values are 10 and 100 times larger than those usually given now.

Biot's experiments led him to believe that the specific rotatory power is a constant property of the molecule. In 1852 Biot described the circumstances which led him to adopt this view in 1836 (Biot, 1852). Since these views are of fundamental importance they are quoted here. "In the numerous experiments which I made between the year 1815 to 1832 to determine the laws according to which molecular rotatory power acts, I worked on organic compounds of mobile structure, sugars, gums, camphors, essential oils, which I was anxious not to alter. This obliged me to dissolve them in inactive and neutral solvents, water, alcohol, ether, fatty oils, which could not modify them chemically, at least during the period covered by the optical observations. In these circumstances the specific rotatory power reduced to unit thickness and unit weight of the active substance was found to be always in the same sense and of the same intensity, whatever the proportion of the inactive solvent which was associated with it. The active molecules thus seemed merely to disperse themselves amongst the inactive molecules, as if in free space without suffering from them any action which modified their rotatory power appreciably. As a result I was able to establish the physical law governing the phenomenon in these simple conditions and the mathematical formula, by which I expressed them, fitted the experiments so well that one could calculate the results in every detail as accurately as they were given by the observations." Experiments carried out in a way where the above mentioned conditions satisfied, showed that $[\alpha]$ the specific rotation was constant within limits of experimental error as it was independent of l (thickness of the column of the liquid) and ϵ (concentration of the active material) and of the nature of the indifferent solvent. Thus these observations tended to establish beyond question the hypothesis that molecules of active substances are scattered in the inactive medium in which they are dissolved as if in empty space without influencing or being influenced by them.

It was statements of the type mentioned above which led scientists to believe at least for a time that optical rotatory power was a physical property by which molecules could be identified and distinguished Inspite of the limitations of the experimental method which led to an accumulation of meagre and not infrequently incorrect data, the next necessary logical step was to try to find how the optical rotatory power varies with different classes of chemical substances. The earliest attempts were of an empirical nature and were handicapped by several factors which influence optical rotatory power which were not known or realized. Nevertheless certain empirical generalizations were obtained and it would be worthwhile to briefly recapitulate them Most of them relate generally to the period before 1914, though some references of later workers are also given to show that interest in such empirical generalizations was continued to even a later date However no references beyond 1925 are given in this context as generally after that date there was increasing realization that such haphazard work has little value a realization which became firmer in 1930 when Kuhn s work was published.

Homologous Series Homologous series naturally attracted the attention of the first workers. In this connection an excellent review of the earlier work has been given by P F Frankland in 1912 to which the reader is referred for a more comprehensive treatment (Frankland 1912)

Tschugaeff studied menthyl esters of aliphatic acids and Guye and Chavanne (1895) esters of active amyl alcohol. They found that the active group is mainly effected by the group in its vicinity and that as one proceeds in the homologous series the value of the active ester drops to a constant. A reverse effect of the phenyl group was noticed by Rupe and Wolfsleben (1912[a]) in the esters of carvoxime Interesting results were obtained by Pickard and Kenyon (1911 1912 [b] 1913 Clough 1913) who studied three series of active secondary alcohols viz. Me R-carbinols Et R-carbinols and isopropyl R-carbinols, where R is alkyl The behaviour of the three series of alcohols was not found to follow similar pattern. If in the first (methyl) series the rotations increased regularly with molecular weight in the second (ethyl) sudden increments were at 5 10 15 carbon radicals and in the third (isopropyl) the rotation increased rapidly upto $R = C H_{\mathfrak{g}}$ and then remained nearly constant The sudden increments at 5 10 15 carbon radical could be explained by the view of Frankland (1899) expressed earlier The explanation suggested was According to commonly accepted view of stereochemistry a continuous chain of five carbon atoms will all but return upon itself and beyond this, further additions to the chain will lead to such interference as must necessitate a readjustment of the exact positions occupied by the carbon atoms in a shorter chain. It is surely highly probabl that this stereochemical change should be betrayed by some irregularity in the rotatory manifestations, fo example, by the exhibition of a maximum rotation in these series in which the ascent of the series leads to an increase in rotatory power Later when these workers (Frankland, 1913) reviewed the whole position in regard to relation of the

numerical value of rotatory power obtained for different members of the homologous series concluded that there does not appear to be any simple relationship in molecular rotatory power of any homologous series.

Unsaturation It was found by Rupe that the presence of unsaturation generally enhances the rotation (Rupe 1903 1909a 1910 1912 b 1913 b 1915 1917) Hilditch (1908b d 1909b 1910 b 1911b) studied menthyl esters and brucine salts of a number of acids containing the phenyl group and unsaturated bonds and found that sometimes a lower and sometimes a higher rotation was observed in presence of a double bond A critical review of the problem both by Frankland (1912b) and by Rupe (1914) could lead only to a generalization of the type that unsaturation leads to an irregularity which is not necessarily an increase in rotation Kenyon and Snellgrove (1925) examined a series of aliphatic vinyl carbinols and corresponding saturated alcohols and found that in every case an increase in rotation due to the presence of unsaturation was shown The effect of triple bond was also studied and compared with the effect of double bond (Rupe 1923 1925 b) The effect of acetylene bond does not appear to follow any rule since it sometimes falls below both the ethylenic and saturated compounds and sometimes it exceeds them (Hilditch, 1908 1911)

These studies were also extended to notice the effect of conjugation. Rupe (1909 1915 Rupe and Busolt 1909b) and also Hilditch (1909) found that the effect of the two conjugated linkages is to increase the rotation. This effect was shown, for example in the comparative studies of *laevo*-menthyl esters of caproic, $\alpha\beta$-hydrosorbic, $\beta\gamma$-hydrosorbic and sorbic acids. Rupe (1909 1915b) himself noticed that in the case of *laevo*-menthyl ester of dimethyl sorbic acid an opposite effect is obtained. Generally however conjugation is found to enhance rotation irrespective of the source of unsaturated bonds. Hilditch (1909d 1911e) ascribed high rotation of menthyl esters and brucine salts of oxalic acid as compared with those of other saturated dibasic acids to the conjugation of the group O C—C O and to the group O C—C=CHR in the case of substances obtained by the action of aldehydes on camphor and thujone (Hilditch, 1909) Exceptionally high rotations were observed by B. K. Singh and M. Singh (1920) in the case of 1·4—naphthalene bis-imino-camphor which contains a large number of conjugated groups in a narrow molecular compass. Others (Forster and Thornley 1909 Forster and Seville, 1921) also noticed the enhancement of rotation by increasing the number of conjugated bonds.

It was also noticed that ring closure usually enhanced rotation as anhydrides and lactones generally show a higher rotation than the corresponding acids. Haller and Desfontaines (1905) compared the numerical value of rotatory power of β—methyl adipic esters and of methyl cyclopentanone carboxylic esters to which they can be converted and found in each case an enhancement of rotation A remarkable exception to this general rule is the

case of camphoric anhydride (Singh and Mohanti 1935) which possesses practically zero rotation. All that one can say is that if the ring structure contains the asymmetric carbon atom then generally enhancement of rotation is noticed, otherwise the effect is indefinite

Optical activity of isomers attracted the attention of the early workers Structural isomers do not follow any general rule but position isomers appeared to show some regularity Cohen and his co-workers (1910, 1911) compared menthyl esters of benzoic and substituted benzoic acids. They found that the ortho substituent was most effective and it may either cause an enhancement of rotation or a lowering of rotation and that the effect of the same substituent in m-and p-positions was insignificant Walden (1896) studied active amyl esters of fumaric and maleic acids racemic and meso-tartaric acid and of β— and l-dimethyl succinic acid and found that the effect of introducing stereo or geometrical isomeric radicals is very marked.

The effect of halogens in a series of substituted menthyl esters of the aliphatic acids was examined by Tschugaeff (1902) Cohen (1911) and Hilditch (1912) and they found that in general, introduction of halogen raises numerical value of rotatory power and the effect of the halogens is in the reverse order of their atomic weights.

From the foregoing summary it is apparent that nothing in the way of a useful broad generalization can be drawn in regard to the relation of value of rotatory power to constitution. It may be mentioned here that quite early in such studies attempts were also made to formulate direct connection between optical activity and asymmetry of the molecules. The earliest attempt to formulate a direct connection between optical activity and asymmetry of the molecule were those of Crum Brown (1890) and Guye (1890, 1891 1893 b c 1894 1895 b d etc.) According to Crum Brown each of the radicals possess a function K which determines its rotation and the rotation of the whole molecule will be given by differences between these functions. Guye asserted that the amount of asymmetry is determined by the displacement of centre of gravity of the regular tetrahedron from its six planes of symmetry Thus the product of the asymmetry (P) assuming the tetrahedron to be regular can be estimated to a first approximation from the differences of the masses of the four radicals (m_1 m_2, m_3, m_4) located at the summits. If $m_1 > m_2 > m_3 > m_4$ then

$$P = \frac{(m_1-m_2)(m_1-m_3)(m_1-m_4)(m_2-m_3)(m_2-m_4)(m_3-m_4)}{(m_1+m_2+m_3+m_4)^6}$$

Expression of this type clearly shows that if two groups are identical, $P=O$ or in other words the substance becomes inactive also, if two groups are interchanged P would become negative which would mean a change of sign. Guye (1893) compared forty three compounds from active amyl alcohol and found in general a good agreement with the theory Soon, however he discovered that two different groups of equal mass do not destroy optical activ

Purdie (1895) refuted Guye's hypothesis with the study of ethoxy and propoxy succinic acids and Walden (1894 1895a b) subjected it to a scathing and searching criticism in his study of active derivatives of substituted malic esters, mandelic esters and succinic esters. Fisher and Flatau (1907) resolved into active components propyl-isopropyl-cyan-acetic acid which contained two groups propyl and isopropyl of close similarity and equal mass. Studies of this type clearly showed that the masses of the radicals are not of primary importance in relation to optical activity

The early position in regard to optical rotatory power has been succintly reviewed in a symposium on Optical Rotatory Power' held by the Faraday Society in 1914 In this symposium in his opening address Armstrong stated In the past apparently workers have dealt too much with substances such as tartrates which presumably can occur in more than one form In the future we must deal more with substances of *known configuration* which are not subject to change, *if there be such*—if we are, to solve problems such as have been stated by Crum Brown and by Guye and correlate rotatory power with structure. This concept of Armstrong was not anything new as Drude Cotton and Tschugaeff has emphasized it earlier and several investigators whose work has just been briefly reviewed tacitly recognized its importance

Further work in this direction proceeded in having increasing realization of the influence of the factors such as solvent, concentration temperature, wavelength of light used and chemical changes if any in trying to base the origin of optical activity on the electromagnetic theory of light and consequently on the electrical character of groups in any scheme of optical activity and chemical constitution

REFERENCES

1 Armstrong *Trans Farad Soc* 10 41 (1914)
Bi *Mem. I I* I 1 372 (1812 *Bull Soc Philomath*, 190-192 (1815) *Mem Acad Sci.*, 2, 41 136 (1817) 13 39-175 (1835) 15 93-279 16 229-396b (1838) *A.C.P* 36, 258 *et seq* (1852)

3. Clough, *Proc.*, 29 357 (1913)

4 Cohen and co-workers, *Trans Chem Soc* 97 1737 (1910) 99 1061 (1911)

5. Crum Brown, *Proc. Roy Soc Edinb* 17 181 (1890)

6. Fischer and Flatau, *Ber* 42. 2981 1909)

7 Forster and Thornley *Trans. Chem Soc* 95, 942 (1909)

8. Forster and Saville *Trans Chem Soc* 119 791 (1921)

9. Frankland, *Trans, Chem. Soc* 75 368 1899) 101 658 661b (1912) 103 1930 (1913)

10. Guye *et al. Compt rend* 110 714 (1890) 111 745 (1891) ; 116, 1133 1378b 1451c 1454d 1893) *Ann Chim Phys.*, 25 146 (1893) *Compt rend* 119 906 (1891) 120, 157a, 452b 632 1274d (1895 etc

11 Haller and Desfontaines, *Compt rend.*, 140 1205 (1905

12. Hilditch *et al Trans Chem Soc* 93, 1 700b 1338 1618d (1908) 95 33 280b 1570c, 1578d 1909 97 223 1091b 2110c (1910) 99 46 218b 224c (1911) 101 192 (191

13. Kenyon and Snellgrove *Trans Chem Soc* 127 109 (1925)

14 Pickard and Kenyon, *Trans Chem Soc* 99 45 (1911) 101 620 1427a (1912) 103 1923 (1913)

15. Purdie *Trans Chem Soc* 67 916 1895)

16. Rupe et. al, *Annalen* 327, 157 (1903); 369, 311, 318[b], 344[c] (1909); 375, 1 1 (1910); 395, 87[a], 136[b] (1912); 398, 372 (1913); 402, 149[b] (1913); *Trans. Farad. Soc.*, 10, 5 (1914); *Annalen*, 409, 327[a], 330[b], 345[c] (1915); 414, 99 (1917); 436, 184 (1923); 442, 61, 74[b] (1925)
17. Singh and Singh, *Trans. Chem. Soc.*, 117, 1599 (1920)
18. Singh and Mahanti, *Proc. Ind. Acad. Sci.*, 2A, 378 (1935)
19. Tschugaeff, *Jour. Russ. Phys. Chem. Soc.* 34, 606 (1902)
20. Walden, *Zeit. Phys. Chem.* 15, 638 (1894); 17, 245[a], 705[b] (1895); 20, 377 (1896)

Purdie (1895) refuted Guye's hypothesis with the study of ethoxy and propoxy succinic acids and Walden (1894, 1895a, b) subjected it to a scathing and searching criticism in his study of active derivatives of substituted malic esters mandelic esters and succinic esters. Fisher and Flatau (1902) resolved into active components propyl-isopropyl-cyan-acetic acid which contained two groups propyl and isopropyl of close similarity and equal mass. Studies of this type clearly showed that the masses of the radicals are not of primary importance in relation to optical activity

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REFERENCES

1 Armstrong *Trans Farad. Soc* 10 44 (1914)
2 Biot, *Mem Inst.* 1 1 37? (1812) *Bull Soc Philomd* 190-192 (1815); *Mem. Acad. Sci.*, 2, 41 136 (1817) 13 39-175 (1835) 15 93- 79 16, 229-396b (1838) *A C P* 36 .58 *et seq* (1852)
3. Clough, *Proc.*, 29 337 (1913)
4 Cohen and co-workers, *Trans Chem Soc* 97 1737 (1910) 99 1061 (1911)
5 Crum Brown, *Proc Roy Soc Edin* 17 181 (1890)
6. Fischer and Flatau, *Ber* 42. 2981 (1909)
7 Forster and Thornley *Trans. Chem Soc* 95 942 (1909)
8. Forster and Seville *Trans Chem Soc* 119 791 (1921)
9. Frankland, *Trans Chem. Soc* 75, 368 (1899) 101 658 661b (1912) 103 1930 (1915)
10. Guye *et al. Compt rend.* 110, 714 (1890) 111 745 (1891); 116, 1133 1378b 1451c 1454d 1893) *Ann Chim. Phys* 25, 146a (1893) *Compt. rend* 119 906 (1891); 120 157a, 452b 63 1274d 1895) etc.
11 Haller and Desfontaines, *Compt rend.*, 140 1205 (1905)
12. Hilditch *et al Trans Chem Soc* 93, 1 700b 1338 1618d (1908) 95 35 289b 1570c 1578d 1909 97 .23 1091b 2110e (1910); 99 46a 218b 224c (1911); 101 ?? (191
13. Kenyon and Snellgrove *Trans Chem Soc* 127 109 (1925)
14 Pickard and Kenyon, *Trans Chem Soc* 99 45 (1911); 101 620a 1427b (1912); 103, 1923 (1913)
15. Purdie *Trans Chem Soc* 67 91? 189)

NUTRITIONAL REQUIREMENTS OF *STREPTOMYCES GRISEUS* AGRA STRAIN ON THE PRODUCTION OF A FUNGISTATIC SUBSTANCE
II NITROGEN SOURCES

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Introduction

Basu Chaudhary reported (1959) the isolation of a new strain of *Streptomyces griseus* identified as Agra strain and found it antagonistic to *Alternaria solani* and a few other fungi and bacteria. The antagonism is perhaps due to another substance other than streptomycin as the latter is not antagonistic to the test fungus. The author has studied the effect of different carbon sources on the production of the fungistatic substance by this strain of *S. griseus* (1961) and found that glucose was the best carbon source. Actinomycetes usually are able to utilize a variety of nitrogen sources for their metabolism. Proteins, peptones and various amino acids form good sources of nitrogen. Lieske (1921) found that urea was an excellent source while asparagine was scantily utilized. Among the inorganic compounds nitrates and ammonium salts are well utilized, the former are sometimes considered better than the latter. Nitrites when used in very low concentrations are also taken up. The effect of different nitrogen sources on the metabolism of *Streptomyces griseus* and the production of the active substance was made by Waksman and Schatz (1945) Dulaney (1948) Eiser and McFarlane (1948) Spilsbury (1948) Thornberry and Anderson (1948) Cochrane (1950) etc.

This investigation was taken up with the view to find out the effect of different nitrogen sources on the production of the fungistatic substance by *Streptomyces griseus* Agra strain.

Methods and Material

The organism was cultured in a basal medium with the following composition glucose 10 gm., dipotassium phosphate 0·5 gm. magnesium sulphate 0·25 gm., per litre of distilled water to which equal amount of nitrogen was added from different sources. Alanine, glycine, glutamic acid peptone, urea acetamide, asparagine, sodium nitrate, sodium nitrite ammonium sulphate, ammonium chloride, ammonium nitrate were selected to supply nitrogen. 50 ml. of the medium were distributed to flat bottles and autoclaved at 15 lbs pressure for half an hour. The medium was then inoculated from a seven day old culture of *Streptomyces griseus* Agra strain and incubated at about 25°C. The pH of the medium was adjusted at 7·0 before autoclaving. The fungistatic potency was measured at regular intervals of 7 10, 13 and 16 days,

after which the mycelial dry weight was taken and the final pH of the basal medium determined. Five replicates of each treatment were maintained.

RESULTS

Table showing the effect of nitrogen sources on the growth of *Streptomyces griseus* Agra strain and the production of the fungistatic substance in terms of 'SD units (mean of five readings)

Sources	D 7	A 10	Y 13	S 16	Initial pH	Final pH	Mycelial Dry Weight in gm.
Alanine	38.2	64.0	128.0	256.0	7.0	7.3	1.6793
Glutamic acid	32.0	64.0	128.0	256.0	7.0	7.3	1.6730
Glycine	32.0	64.0	128.0	256.0	7.0	6.7	1.6728
Peptone	16.0	32.0	64.0	128.0	7.0	6.4	1.4010
Asparagine	16.0	32.0	64.0	128.0	7.0	6.4	1.4010
Urea	8.0	16.0	32.0	64.0	7.0	5.8	1.0122
Acetamide	8.0	16.0	32.0	64.0	7.0	6.1	1.0123
Ammonium nitrate	8.0	9.6	19.2	32.0	7.0	5.5	0.8250
Ammonium sulphate	7.2	8.0	16.0	16.0	7.0	5.5	0.5201
Ammonium chloride	4.0	5.6	16.0	16.0	7.0	4.0	0.5201
Sodium nitrate	4.0	7.2	8.0	16.0	7.0	5.5	0.4793
Sodium nitrite	0.0	0.0	4.0	4.0	7.0	4.0	0.1974

Observations in the above table indicate that the nitrogen sources can be placed in six groups depending upon the yield of the fungistatic substance in the following descending order: (i) amino acids giving the highest production of 256 SD units and comprising of alanine glutamic acid and glycine (ii) peptone and asparagine group in which the production is 128 'SD units (iii) urea and acetamide yielding 64 'SD units (iv) ammonium nitrate having the yield of 32 SD units and (v) and (vi) comprising of inorganic

salts in which the production of the fungistatic substance is 10 and 4 SD units respectively There is also a similar correspondence with the growth (dry weight) of the organism indicating a direct relationship between growth and the production of the fungistatic substance.

In most cases the initial pH of the basal medium changed to acidic values

Discussion

For cellular activity Actinomycetes are capable of utilizing nitrate and ammonia as sources of nitrogen apart from organic compounds. Streptomycin is best produced on media containing complex organic substances though it is also produced in synthetic media but the process is much slow and the yield is poor Somewhat contradictory views have been expressed about the nitrogen sources. Waksman and Schatz (1945) reported that nitrogen source is non-specific for the production of streptomycin but Thornberry and Anderson (1948) found that nitrate would not support the growth of the organism. In the present experiment as well nitrate nitrogen which has been found to be a good source by Waksman and Schatz (1945) and other workers for many fungi, has not proved so.

The nitrogen requirement of the organism under study resembles the findings of Spilsbury (1948) Elser and McFarlane (1948) on *Streptomyces griseus* that amino acids are far superior sources of nitrogen than the inorganic salts. Peptone which is sometimes considered as the best source of nitrogen has been relegated to the second place along with asparagine Urea and acetamide are utilized no doubt but are not so good

Among the inorganic salts both ammonium salts and nitrate nitrogen appear to be of the same standard though ammonium nitrate has given better yield and growth than sodium nitrate. Ammonium chloride which is considered to be one of the poorest sources and is not utilized by many micro-organisms has given better results and has come up along with sodium salt and ammonium sulphate. Sodium nitrite as usual is not much utilized by the organism and remains the poorest source of nitrogen in this case too.

Summary

This paper deals with the effect of twelve nitrogen sources alanine, glycine, glutamic acid, peptone, asparagine, urea, acetamide ammonium nitrate, ammonium chloride ammonium sulphate sodium nitrate and sodium nitrite—on the production of the fungistatic substance produced by *Streptomyces griseus* Agra strain.

All the nitrogen sources were utilized to varying degrees, the order being (i) alanine, glycine and glutamic acid (ii) peptone and asparagine (iii) urea and acetamide (iv) ammonium nitrate (v) ammonium sulphate ammonium chloride and sodium nitrate and (vi) sodium nitrite.

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REFERENCES

1 Basu Chaudhary K C 1959 : Studies on the production of fungistatic substance by certain soil fungi *Ph D Thesis, Agra University*

2 Basu Chaudhary K C 1961 : Nutritional requirements of *Streptomyces griseus* Agra strain on the production of a fungistatic substance—I Carbon sources. *Proc. Nat Acad Sc India* B 31 : 268-272.

3 Cochrane, V W 1950 : The metabolism of species of *Streptomyces* III The nitrate metabolism of *Streptomyces griseus* *Bull Torrya Bot Club* 77 : 10-18

4 Dulaney E L 1948 Observations on *Streptomyces griseus* II Nitrogen sources for growth and streptomycin production *Mycologia* 6 305-314

5 Eiser H M & McFarlane, W D 1948 Metabolism of *Streptomyces griseus* in relation to production of streptomycin *Canad J Res C* 26 164-173

*6 Lieske R 1921 Morphologie und Biologie der Strahlenpilze Leipzig

7 Spilsbury J F 1948 : Observations on the nutritional requirements of *Streptomyces griseus* (Krainsky) Waksman & Schatz *Trans Brit Mycol Soc* 31 10-228

8 Thornberry H H & Anderson, H W 1948 Synthetic medium for *Streptomyces griseus* and the production of streptomycin *Arch Biochem* 16 : 389-393

9 Waksman S A & Schatz, A 1945 Streptomycin—origin, nature and properties *J Amer Pharm Assoc Sc Ed* 34 273-291

*Original not seen.

PHYSIOLOGICAL STUDIES ON SALT TOLERANCE OF CROP PLANTS
V USE OF IAA TO OVERCOME DEPRESSING EFFECT OF SODIUM SULPHATE ON GROWTH AND MATURITY OF WHEAT

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INTRODUCTION

The problem of saline and alkaline soils is of vital importance in many countries including India, as due to excessive salinity or alkalinity large tracts of arable land are rendered barren further even under moderate conditions growth and maturity of crop plants are considerably reduced Soil salinity is attributed to a high concentration of soluble salts, particularly chloride and sulphate of sodium, and is known to lead gradually to alkaline condition. Reclamation of such soils is attempted with varying success by agronomical methods and by growing salt or alkali tolerant crops and varieties In this laboratory work has been in progress since some years on problems relating to salt or alkali tolerant crops and varieties Independantly of these studies, investigations have been carried out on the influence of synthetic phytohormones on growth and maturity of crop plants grown in normal soil. As results of the latter studies proved promising, it was felt that the beneficial influence of growth hormones could be utilised in overcoming the adverse effect of sodium salts on growth and maturity of crop plants. The present paper deals with the results of an investigation on these lines, carried out on wheat in pot-cultures during three cropping seasons (1955 1958)

In the alluvial soil of the Gangetic basin (India) the concentration of sodium sulphate varies from 0 026 % (on oven-dry basis) in normal soil to 0 269 in saline or alkaline soils (Desai and Sen 1953) The depressing influence of sodium sulphate on growth and yield of various crop and vegetable plants has been repeatedly established by various workers (Lyon, 1941 Magistad *et al* 1943 Hayward and Long 1942 and 1943 Gauch and Wadleigh, 1945 and Bhardwaj and Rao, 1955) On the other hand the stimulating effect of phytohormone treatment on the growth and maturity of crop plants in normal soil has been observed by many authors (Cholodny 1936 Mc Rostie *et al* 1938 Amlong and Naundorf 1939 Hopkins 1940 and Bhardwaj and Rao, 1955 and 1956)

The use of phytohormones to recover the depression in growth of plants in saline soils has not so far been reported by others. In the present study an attempt has been made to investigate the possibility of nullifying the adverse effect of sodium sulphate in soil on growth and yield of wheat by presowing soaking of the seeds in a suitable concentration of IAA (β-indolylacetic acid)

Investigations carried out at the Department of Botany Agra College, Agra, India.

The selection of IAA and its concentration as well as of the harmful concentration of sodium sulphate were based on the results of separate investigation by the author (1959) on seedling growth.

METHODS AND MATERIAL

Seeds of wheat Pb C.591 were kept in petri-dishes half-immersed in a solution of 5 ppm. of IAA and in distilled water for 24 hours at 28°C and were sown in first week of November in earthen pots, each containing about 8 lbs. of air-dry soil with compost (1 1) untreated seeds were sown a day earlier Two series were maintained (i) with sodium sulphate at 0 1% (on air-dry-weight of the soil) in 1955-56 and 0 15 % in 1956-57 and 1957 58 the salt was added to the pots in solution immediately after sowing necessary precautions were taken to collect the percolating soil solution from the pots and to add it back to the same and (ii) normal soil without any added sulphate. Four pots were kept for each treatment in the first year and three pots in the following years.

Ten seeds were sown per pot and four weeks after sowing, the seedlings were thinned to three. Monthly observations on the growth of plants were recorded till maturity but in the present paper data on the shoot-dry-weight at maturity and yield of grain is considered for sake of brevity The results are subjected to statistical analysis following the analysis of variance method on factorial basis, with 12 and 9 replications (3 plants each in 4 pots during 1955-56 and in 3 pots during 1956-57 and 1957 58 respectively) The degrees of freedom are distributed as

Salt levels in soil (1 without sodium sulphate—normal soil 2. with added sodium sulphate)	1
Seed treatments (1 control untreated seeds 2 seeds soaked in distilled water 3 seeds soaked in IAA)	2
Salt levels × Seed treatments	2
Residual (in 1955-56)	66
Residual (in 1956-57 and 1957 58)	48

The values for critical (significant) difference at 5 probability are given for only the statistically significant factors and their interaction in the table included under results.

RESULTS

The results are summarised in Table I

1955-56 experiment

It may be noted that sodium sulphate was added to the soil at 0 1% (on air-dry weight of the soil) in this experiment For both the observations, shoot-dry weight at maturity and yield of grain neither the factor 'salt levels nor the interaction was significant. The factor 'seed treatments' was however significant with a critical difference of 0.22 (for shoot-dry weight) for the average values of each treatment (average of the two salt levels) and of 0.21

Table 1

Influence of IAA on the depressing effect of sodium sulphate on growth and maturity of wheat.

Season	Normal soil			Na_2SO_4 added soil			C. D. at 5%		
	Cont.	D. W.	IAA	Cont.	D. W.	IAA	S. L.	S. T.	I. t.
1955-56	Shoot-dry-weight in gms.								
	1·15	1·22	1·55	0·98	1·14	1·42	—	0·22	—
	Yield of grain in gms.								
	1·09	1·44	1·75	1·19	1·16	1·76	—	0·24	—
1956-57	Shoot-dry-weight in gms.								
	2·87	2·42	1·62	1·89	0·79	2·49	—	—	1·07
	Yield of grain in gms.								
	2·43	2·32	1·59	1·63	0·91	1·94	0·38	—	—
1957-58	Shoot-dry-weight in gms.								
	1·64	2·27	2·70	1·81	2·66	3·13	—	0·62	—
	Yield of grain in gms.								
	1·29	1·44	0·82	0·98	0·94	1·23	—	—	0·56

Cont. -Control (untreated seed.)
D. W. Distilled water soaked seeds.
IAA IAA soaked seeds.
S. L. Salt-levels ('Main factor')
S. T. Seed-treatments ('Main factor')
Int. -Interaction ('Salt-levels x 'Seed-treatments)
C. D. -Critical difference

for yield of grain. Thus, although the depression in growth of plants (as indicated by shoot-dry-weight at maturity) due to sodium sulphate (0·1% concentration) was clear the difference was small and statistically non-significant apparently the concentration of the salt was low the seed-treatments, particularly IAA soaking significantly increased the growth of the plants compared to those from control (untreated seeds) or even from seeds treated with water only

Regarding grain-yield, the saline soil in general seemed to be beneficial, unlike the adverse effect on growth. Seed-treatments, particularly with IAA, increased the yield of grain significantly over the control set in both the normal and saline soil

Since the interaction was not significant both for shoot-dry weight and grain-yield, it is clear that each of the two factors operated separ tely

In the experiment of the following years, 1956-57 and 1957 58, the amount of sodium sulphate added to the soil, was raised, bringing the concentration to 0·15% (on air-dry-soil) in order to obtain depression in growth and maturity which was absent with 0·1% concentration in the earlier experiment.

1956-57 experiment

During this season Control (Untreated seeds') plants in normal soil were unfortunately damaged leaving only three replications, in stead of nine as in the remaining treatments, and these plants grew relatively vigourously as seen in the shoot-dry-weight at maturity and yield of gram further the variation between plants was high, which increased the error variance and also the critical difference for the significant factor or interaction Still the trends for the treatment effects in the normal and saline soil are interesting

Regarding shoot-dry weight at maturity the factor salt level showed differential effect under different seed treatments thus the general effects of the two factors became statistically non-significant while their interaction was significant.

In the normal soil (0 % salt level) seed treatment with IAA decreased the growth compared to its control (untreated seeds) but as already pointed out, the few available control plants were unusually vigorous In the saline soil IAA resulted in an apparent increase in growth over its respective control, the values being 2 49 gms. and 1 89 gms. of shoot-dry-weight per plant respectively Soaking the seeds in water alone proved harmful to growth, definitely so in the saline soil

The grain yield, unlike the shoot-dry-weight, was in general depressed in saline soil (compared to the yield in normal soil) as indicated by the statistical significance of the factor effect (salt-level) further as in the shoot-dry weight, the interaction was also significant indicating the differential influence of the seed-treatment on grain-yield of the plants in the two soils. The trends for the treatment-effects on yield were similar to those observed for growth (shoot-dry weight)

1957 58 experiment

The sulphate concentration of the soil was the same (0 15) as in the previous year There was no damage to any of the plants during this season. It is interesting to note that even 0 15 % of the sulphate tended to improve in general, the growth of the plants but the effect was, however not statistically significant. The seed-treatments, particularly IAA, significantly improved the growth in both the soils compared to the respective controls (untreated seeds) Grain yield was lowered in the sulphate soil for plants from the untreated seeds, compared to the yield in the normal soil It was also lowered by IAA but only in the normal soil in saline soil the yield was enhanced by IAA treatment from 0 98 gms. (untreated set) to 1 23 gms. (IAA set)

Discussion

Inspite of the fact that the studies were carried out during three cropping seasons the results show apparent variation in the effect of the sodium sulphate

or of the seed-treatments in the different seasons. It is not uncommon particularly with presowing seed-treatments (Bhardwaj and Rao 1955 and 1956). However the general trends are clear and become more evident from the following discussion.

As already mentioned in the review of literature, sulphate injury to plants resulting in depressed growth was reported by several workers. It was also noted in seedling growth of wheat and gram by the author (Sarin 1959). In sand culture too, growth and yield of wheat plants was lowered by a supply of sodium sulphate in the culture solution (Sarin, 1959). But in the present study where sodium sulphate was added to the soil in fairly high concentration (0.1 and 0.15%) the adverse effect on growth and yield, was not consistent in the three years. However the average values including the results of 1955-56 when a lower concentration of the sulphate was tried indicate clearly the depressing effect of the salt.

Table 2

Percentages of the values for the 'saline soil' over those for 'normal soil'

(Plants from untreated seeds)

Season	Shoot-dry weight %	Yield of grain %
1955-56	86.7	109.1
1956-57	65.8	67.0
1957-58	110.5	75.9
Mean	87.6	84.0

The figures for 1955-56 relate to 0.1% sodium sulphate while in the other two years, it was 0.15%. The figures for 1956-57 are apparently too low due, as has already been explained to the unexpected better growth of the few undamaged plants of the control set in the normal soil. The causes for absence of the adverse effect of the salt on (or even for the apparent improvement) the yield of grain in 1955-56 and in shoot growth in 1957-58, are not clear.

According to Cholodny (1936), Hopkins (1940) and others synthetic phytohormones improve growth and maturity of plants, although Barton (1940), Stewart and Hamner (1943) and others contradict the same. Presowing seed treatments with hormones gave better growth and yield of wheat but the results varied in different years (Bhardwaj and Rao 1955 and 1956). In the present study too, considering the effect of IAA treatment in the normal soil, it was variable although the beneficial effect was clear in some years. However the main object of the present investigation was to note the effect of IAA on plants growing in the saline soil which is clear from the following figures —

1956-57 experiment

During this season Control (Untreated seeds) plants in normal soil were unfortunately damaged leaving only three replications, in stead of nine as in the remaining treatments, and these plants grew relatively vigourously as seen in the shoot-dry-weight at maturity and yield of grain further the variation between plants was high, which increased the error variance and also the critical difference for the significant factor or interaction Still the trends for the treatment effects in the normal and saline soil are interesting

Regarding shoot-dry weight at maturity the factor salt level showed differential effect under different seed treatments thus the general effects of the two factors became statistically non-significant while their interaction was significant.

In the normal soil (0 % salt level) seed treatment with IAA decreased the growth compared to its control (untreated seeds') but as already pointed out, the few available control plants were unusually vigorous in the saline soil IAA resulted in an apparent increase in growth over its respective control, the values being 2 49 gms. and 1.89 gms. of shoot-dry-weight per plant respectively Soaking the seeds in water alone proved harmful to growth, definitely so in the saline soil.

The grain yield unlike the shoot-dry-weight, was in general depressed in saline soil (compared to the yield in normal soil) as indicated by the statistical significance of the factor effect (salt-level) further as in the shoot-dry-weight, the interaction was also significant indicating the differential influence of the seed treatment on grain-yield of the plants in the two soils. The trends for the treatment-effects on yield were similar to those observed for growth (shoot-dry weight)

1957 58 experiment

The sulphate concentration of the soil was the same (0.15) as in the previous year There was no damage to any of the plants during this season It is interesting to note that even 0 15 % of the sulphate tended to improve in general, the growth of the plants but the effect was, however not statistically significant The seed treatments, particularly IAA, significantly improved the growth in both the soils, compared to the respective controls (untreated seeds) Grain-yield was lowered in the sulphate soil for plants from the untreated seeds compared to the yield in the normal soil It was also lowered by IAA, but only in the normal soil in saline soil, the yield was enhanced by IAA treatment from 0 98 gms. (untreated set) to 1·25 gms. (IAA set)

DISCUSSION

Inspite of the fact that the studies were carried out during three cropping seasons the results show apparent variation in the effect of the sodium sulphate

as those from untreated seeds in normal soil i. e., can the depressing effect of the salt be overcome completely by the beneficial effect of the hormone? This aspect become clear from the following table

Table 5

Percentage of IAA-treated set in saline soil over untreated set in normal soil.

Season	Shoot-dry-weight %	Yield of grain
1955-56	125·6	161 4
1956-57	86 7	79 8
1957 58	190·8	96·9
Mean	134 3	112 7

Although the conclusion is evident and attractive, regarding the utility of the hormone treatment of plants in the saline soil, the author do realise that it is as yet only of academic importance but he is confident that it opens out a new line of investigation for the improvement of crop growth in saline soils.

Finally would it be possible to visualise the mechanism of sulphate injury from the present data which, however relate only to the final performance of the plants. According to Magistad et al (1943) and others it is merely an osmotic phenomenon Other workers (Eaton, 1942 etc.) however attribute the injury to sulphate toxicity The fact that sulphate depressed the total growth and yield of wheat plants and that the growth promoting substance, IAA, neutralised the adverse effect can indicate the injury to be primarily an osmotic effect. IAA is known to increase root-elongation inspite of the objections raised by Aberg (1957) who convincingly analyses the various aspects of the IAA-effect on root-elongation. IAA supplied at 5 ppm. in sand cultures, increased significantly the total root growth in potato plants (Bajal and Rao, 1958) Thus, if the growth promoting activity of IAA is accepted, it may then mean that, in the present study the depression in growth of wheat plants due to the sulphate might be an osmotic effect which was neutralised by the IAA. It received further support from the data on the favourable effect of IAA in neutralising the retarding effect of sodium sulphate on root and plumule growth of wheat seedlings (Sarin, 1959) One doubt arises, however in this connection. Can the presowing seed-treatment with hormones exert a long duration effect? The literature on the effect of hormones, used as spray or seed-treatments, gives convincing proof of the continued effect of the treatment, even upto maturity

Inspite of the above arguement in support of the osmotic injury due to the sulphate, it appeared that it may have toxic effect too as reduction in growth and yield in wheat plants due to sulphate were neither related to each other nor

consistent in the present investigation. All the same, IAA could apparently overcome even the toxic effect, if any of the sulphate. Thus it many once again be pointed out that, if a growth promoting substance like IAA can neutralise the sulphate injury to growth and maturity of plants, it is worthwhile investigating the salinity problems with more powerful growth hormones like gibberellic acid, the wonder chemical which is as yet not easily available to all workers.

Abstract

Local saline and alkaline soils contain a fairly large amount of sodium sulphate even upto 0 269% (on oven-dry soil) Reduced crop growth or even complete failure is a common feature in such areas. The present study was undertaken to find out the adverse influence, if any of a fairly high concentration of sodium sulphate on growth and maturity of wheat and to evaluate the possibility of its neutralisation by treatment with the growth promoting substance, IAA.

Seeds of wheat Pb C. 591 were subjected to presowing soaking (bal immersed) in 5 ppm. of β-indolylacetic acid (IAA) solution and in distilled water for 24 hours and were sown in pots along with untreated seeds. Two series were maintained for each treatment (1) with 0 1% (in 1955-56) and 0 15% (in 1956-57 and 1957 58) of sodium sulphate added to the soil and (2) normal soil without any added sulphate The experiment was conducted during three years i e., in rabi (winter cropping season-October to April) of 1955-56, 1956-57 and 1957-58

The lower dose of sodium sulphate (0 1% on air-dry weight of the soil) did not decrease the shoot-dry-weight and yield of grain but the higher dose (0 15) was definitely inhibitive the average reduction in shoot-dry weight and yield of grain due to the sulphate for the three years amounted to nearly 12 and 16 % respectively

Presowing soaking of wheat seeds in IAA solution increased on an average the shoot dry weight by 19 % but scarcely affected the yield when the plants were grown in normal soil. In the sulphate soil however IAA treatment increased significantly shoot-dry weight and grain yield of the plants by 50 and 31 respectively over the untreated set further plants from the IAA treated seeds, grown in the sulphate soil, showed even better growth and yield than those from untreated seeds sown in normal soil the average increase in the shoot dry-weight and yield of grain being 34 and 13 % It clearly shows the utility of IAA treatment to overcome the depressing effect of the sulphate in the soil on growth and maturity of wheat plants. Presowing soaking of the seed in distilled water gave erratic results in the sulphate soil it was definitely harmful to the yield of grain

The mechanism of sulphate injury is discussed and the use of synthetic phytohormones in crop husbandry in saline soils is advocated.

References

1 Aberg, B. (1957) Auxin relations in roots. Ann. Rev. Plant Physiol., 8 153-180
2. Amlong, H. U and Naundorf, G. (1939) Wuchsstoff und Pflanzenertrag Untersuchungen uber die Wirkung zusätzlicher Wuchstoffgaben auf Keimung, Entwicklung und Ertrag land-wirtschaftlicher und gärtnerischer Kulturpflanzen Forschungsdienst (Neudamm)7 463-482
3. Baijal, B D and Rao, I M. (1958) Effect of preseaving treatment of seed potatoes with synthetic hormones on yield of tubers Modern Developments in Plant Physiology Ed. P Maheshwari 5-
4 Barton, L. V (1940) Some effects of treatment of non dormant seed with certain growth substances. Con Boyce Thompson Inst., 11 181 205
5. Bhardwaj, S.N and Rao, I.M. (1955) Studies on the effect of chloride and sulphate of sodium on germination, growth and maturity of gram. Agra Univ Jour Res S) 4 (Suppl.) 767 776
6. Bhardwaj, S.N. and Rao, I.M. (1955) Studies on the effect of 2,4-D NAA and IAA on growth and maturity of wheat. Jour Ind Bot. Soc 34 21 78.
7 Bhardwaj S N and Rao, I.M (1956) Influence of time of sowing on the effect of 2,4-D on growth and maturity of wheat. Physiologia Plantarum, 9 257 264
8. Cholodny N G (1936) Hormonization of grains. C. R Acad. Sci., U S. S R., 3 439-442.
9. Desai, S.V and Sen, A. (1953) Studies on the characteristics of saline and alkaline patches in the soil of Pusa. Ind Jour Agric Sci., 23 187 202
10 Eaton, F M. (1942) Toxicity and accumulation of chloride and sulphate salts in plants. Jour Agr Res 64 357 399
11 Gauch, H.G and Wadleigh, C.H. (1945) Effect of high concentration of sodium, calcium, chloride and sulphate on ionic absorption by bean plants Soil. Sci. 59 139-153
12. Hayward, H. E. and Long E. M. (1942) Vegetative responses of the Elberta peach on Lovell and Shalil root stocks to high chloride and sulphate solutions. Amer Soc Hort. Sci. Proc. 41 149-155.
13. Hayward, H.E. and Long E. M. (1943) Some effects of sodium salts on growth of tomato. Plant Physiol 18 556-569
14 Hopkins, J W (1940) Effect of phytohormone dust seed treatment on growth and yield of barley under greenhouse conditions. Canad Jour Res C 16 510-515
15. Lyon, C. B (1941) Responses of two varieties of tomato and the F1 generation to sodium sulphate in nutrient medium. Bot. Gaz., 103 107 122.
16. Magistad, O C., Ayers,A.D Wadleigh, C.H. and Gauch. H.G (1943)Effect of salt concentration, kind of salt and climate on plant growth in sand cultures. Plant Physiol., 18 151 166.
17 McRostie G P Hopkins, J W and Grace N H. (1938) Effect of phytohormone dusts on growth and yield of winter wheat varieties. Canad. Jour Res. C 16 510-515.
18. Sarin, M.N (1959) Investigations on the effect of sodium sulphate on (a) seedling metabolism and (b) growth and maturity of wheat and gram. Doctoral thesis Agra University
19 Stewart, W S and Hamner C. L. (1942) Treatment of seeds with synthetic growth regulating substances Bot Gaz. 104 338-347

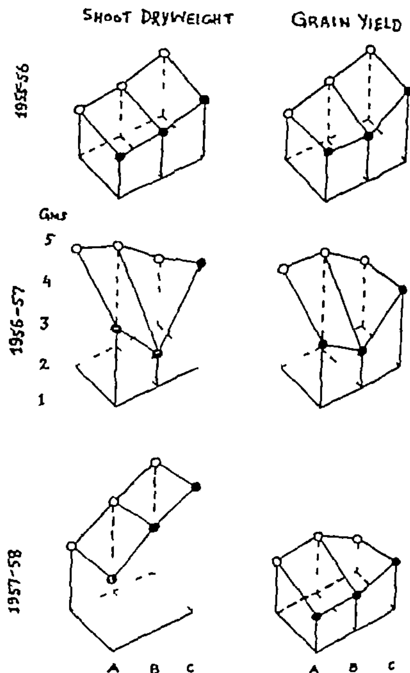


Fig 1 Influence f IAA on the depressing effect of sodium sulphate on growth ond matur ty of wheat

● Plants growing in sodium sulphate added soil

○ Plants gr wing in n rmal soil

A C ntr l (Untreated seeds)

B Distilled water soaked seeds

C IAA soaked seeds.

STUDIES ON THE PATHOLOGY OF BOVINE NEOPLASMS WITH SPECIAL REFERENCE TO HORN CANCER*

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Studies on the pathology of Horn Cancer and other bovine neoplasms from the naturally occurring cases were undertaken to study the problems of metastases in relation to various grades of horn cancer and to set up a probable pathogenesis of this condition. Other neoplasms were studied from the incidence point of view.

Investigations were conducted on 41 horn cancer cases and 13 cases of other bovine neoplasms.

Bending of the horn is the characteristic symptom of the condition by which it is first noted. Slowly the horn completely bends and may even fall down leaving a cauliflower like mass. It has been found under the present investigations that the bending of the horn is associated with destruction of the bony prolongations of the frontal bone at the base of the horn and as the destruction progresses the degree of bending goes on increasing leading to complete drooping of the horn as a result of replacement of bone by cancerous tissue and finally falling down of the horn.

In horn cancer it is the stratified squamous epithelium in between the horny layer and the bone covering the dermal papillae and not the columnar epithelium lining the bony septa internally which proliferates to form the cancer cell nests. This columnar epithelium is the continuation of nasal mucous membrane. After proliferation of the epidermal epithelium towards dermis the proliferation and further formation of the cell nests progresses together with the proliferation of the stroma to support the cell nests. First the cell nests appear as very small aggregations of malignant cells in various irregular sizes and shapes, without any keratinization in the centre. Individual cell keratinization is also noted. Later on when cell proliferation continues the cells in the centre of the pearls start keratinizing due to lack of blood supply. In older pearls keratinization goes on progressing leaving only a narrow rim of malignant cells surrounding a keratinized mass. Finally a mass of concentrically arranged keratinized tissue and fibrous tissue is left.

The proliferating cancer cell masses when come in contact with bone trabeculae the trabeculae are pressed and finally destroyed. Bone trabeculae may be surrounded completely by malignant cells.

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As the sections from various places do not give the same histopathological picture this can be inferred that the proliferation does not occur in whole of the horn at the same time.

Grading of the horn cancer was done as it was a squamous cell carcinoma histologically Grading was done on the basis of histopathological picture. Majority of the horn cancer cases fell into the category of grade I one case fell in grade III category and a few cases fell in grade II No case could be recorded of grade IV type. On the basis of the obtained results of grading the rare records of metastases in horn cancer were accounted for as metastases generally do not occur so long as the cancer is in a grade I stage.

Out of the 13 other bovine neoplasms keratotic form of basal cell papilloma was also recorded from a calf having warts on the body The rest 12 tumours were of the following variety —

(a) Fibroma (Hard)

(b) Nevus verucosus (papilloma)-warts

(c) Nevus verucosus (papilloma)-on udder

(d) Adenoma in the eye probably of lacrimal gland,

(e) Squamous cell carcinoma in eye,

(f) Prickle cell carcinoma of the III eye-lid,

(g) Squamous cell carinoma in mammary gland

(h) Haemangioma,

(i) Melanoma,

(j) Adenocarcinoma of undetermined origin with metastases to liver kidneys lungs and lymph nodes.

STUDIES ON THE MODIFIED ILKOVIC'S EQUATION

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INTRODUCTION

For polarographic diffusion current Ilkovic derived the equation

$$i_d = 607\, m^{\frac{2}{3}}\, t^{\frac{1}{6}}\, n\, C\, D^{\frac{1}{2}} \quad (i)$$

where i_d is diffusion current in microamperes m the mass of the mercury in mgms flowing per second t the drop-time in seconds C the concentration in millimoles of the electroactive species and D its diffusion coefficient (cm.Sec)

Eq (i) suggests that the diffusion current constant, I $(= i_d / C m^{\frac{2}{3}} t^{\frac{1}{6}})$ should be independent of m and t. Lingane and coworkers[2] however observed a marked dependence of I on m and t and suggested the modified Ilkovic's equation[3]

$$i_d = 607\, n\, m^{\frac{2}{3}}\, t^{\frac{1}{6}}\, D^{\frac{1}{2}}\, C\, (1 + A D^{\frac{1}{2}} m^{-\frac{1}{3}} t^{\frac{1}{6}}) \quad (ii)$$

In Eq (ii) all the terms have the same significance as in (i) except A which is a constant. Many attempts have been made to justify theoretically the modification of Ilkovic's equation and to evaluate the constant A[4-7] (vide infra) The present paper outlines an empirical approach for the determination of A by employing the polarographic data of the reduction of O-nitroaniline and its diffusion coefficient determined under actual polarographic conditions.

EXPERIMENTAL

O-nitroaniline sample of Judex Chemicals was recrystallised from double distilled water (M. Pt. 72°C) Sorensen buffers of pH 7 and 8 prepared from Analar samples were used The pH of the experimental solutions was checked with a Beckman H type pH meter which was pre-standardised by standard Beckman buffers of pH 7 0 0 01 solution of gelatin was employed for maxima suppression. Because of the low solubility of O-nitroaniline in water all the experiments were carried out in 25% ethanol water mixtures.

A manually operated polarograph was employed in the present investigations. The details of the circuit and the experimental set up has been described elsewhere The capillary employed had the following characteristics
m=1 463 mgm sec t=2 564 secs.

For determination of the diffusion coefficient of O-nitroaniline under polarographic conditions McBain Dawson cell was used the experimental set up and the procedure has been described earlier in detail.[10] The cell constant

of the cell determined by employing KCl as the diffusing species corresponded to 3 132 The amount of O-nitroaniline diffused was determined spectrophotometrically The spectrophotometric measurements were made with a Beckman DU spectrophotometer using 1 Cm cells of silica and corex for studies in the ultra violet and visible region of the spectrum respectively

Results and Discussion

Examination of the spectra of O-nitroaniline solutions identical in composition with those employed in polarographic investigations indicated the existance of two absorbance maxima at $\lambda=280$ and 420 mμ[11] Beers law was applicable at these wavelengths. The data on the variations of the optical density with concentration of O-nitroaniline gave a value of 4.93×10^{3} and 5.81×10^{3} for the extinction coefficient of nitroaniline at $\lambda=420$ and 280 mμ respectively

In Table 1 are presented the results of the experiments on the determination of D of O-nitroaniline. Column 2 Table 1 gives the values of known intervals of time for which the substance was allowed to diffuse. The amounts of O-nitroaniline diffused in different time intervals obtained from the measured optical density (column 3) and the known values of the extinction coefficients of O-nitroniline (see above) are given in column 4 Table 1 The values of the diffusion coefficient were obtained by the application of King and Cathcart equation[12]

$$\beta DT = \frac{V}{V} \frac{V}{+V} \log \frac{Co}{Co-\left(1+\frac{V}{V}\right)C}$$

where β is the cell constant V and V the volumes of the upper and lower compartments of the diffusion cell (171 8 and 174 4 c c. respectively) Co the initial concentration and C the concentration diffused in time T The values of D obtained from the above equation are returned in column 5 Table 1 These suggest a value of 5.92×10^{-6} $Cm^{2}\ Sec^{-1}$ and 7.08×10^{-6} $Cm^{2}\ Sec^{-1}$ for D of O-nitroaniline in the systems with pH 7 and 8 respectively These values are of the same order of magnitude as obtained by earlier workers in pure ethanol solutions[13]

Table 2 summarises the results on the evaluation of the constant A of the modified Ilkovic's equation using the experimentally determined value of D In column 1 and 2 are given the values of the concentration of O-nitroaniline and the corresponding diffusion currents. Column 3 gives the values of A deduced from the known values of all the factors of Eq (ii) and using a value of 6 for n It is of interest to note that the data in Table 2 suggest an average value of 31 21 for A In t [illegible] is the comparison of the value with those obtained by earlier workers from different considerations and summarised in Table 3

The value of 39 for A obtained by Lingane and Loveridge is based on the consideration of expansion of the mercury drop as well as the curvature of the electrode surface. The so called impoverishing effect[11,15] has been taken into account by Stackelberg[3] whose value for A viz 17 is generally considered too low. Further the suggestion of Stakelberg and also of Meites and Meites[16] to substitute the factor 607 in Ilkovic's equation by 619 and 560 respectively is not considered justifiable[17,18]. It has, however been shown recently that Matsuda has formulated the best mathematical approach and has presented from theoretical considerations a definite solution of the problem. It is of interest to note that the value of A (31.21 ± 2.27) obtained in the present investigations is in close agreement with the value of 31.7 suggested by Matsuda.

Summary

The modified Ilkovic's equation for polarographic diffusion current—

$$i_d = 607\ n m^{2/3} t^{1/6} D^{1/2} C (1 + A D^{1/2} m^{-1/3} t^{1/6})$$

has been examined employing irreversible polarographic reduction of O-nitroaniline. The diffusion coefficient of the substance has been determined under polarographic conditions using a McBain Dawson cell. From the experimentally determinable factors of the modified Ilkovic's equation the value of the constant A has been deduced empirically. A value of 31.21 ± 27 has been obtained for the constant A in close agreement with theoretically justified value of 31.7 obtained by Matsuda.

Acknowledgement

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Table 1

Diffusion coefficient of O-nitroaniline under polarographic condition

pH	Initial concentration of O-nitroaniline mM	Time of diffusion Hours.	Concentration of O-nitroaniline diffused mM	D Cm. Sec. ×10⁶
	0.8	72.5	0.04852	5.895
6.90	1.0	69.0	0.05435	5.775
	1.2	55.8	0.05725	6.078
	0.8	84.5	0.06545	6.980
8.06	1.0	84.0	0.07620	7.331
	1.2	58.0	0.06858	6.918

Table 2

Computation of the constant A of the Modified Ilkovic's Equation

pH	Concentration of O-nitro-aniline mM	Diffusion current μA	A
	0 4	5 75	32 214
6 90	0 6	8 65	33 473
	0 8	11 51	3? 577
	1 0	14 40	32 971
	0 4	6 30	30 19?
8 06	0 6	9 42	28 911
	0 8	12 62	30 796
	1 0	15 180	31 470

Average value of A=31 207 ± 2 666

Table 3

Values of the Constants A obtained by Different Workers

Lingange and Loveridge	30 0
Stackelberg[b]	17 0
Kambara and Tachi	29 3
Koutecky[d]	31 0
Meites and Meites	29 0
Matsuda[f]	31 7
Macero and Rulfs[g]	31 3
Present Investigation	31 21 ± 2 ?7

(a) ref. 3; (b) ref. 5; ( ) ref. 7 (d) ref 19; ( ) ref 16 (f) ref 6; (g) ref 18

References

1. Ilkovic, 1934, Coll. Czech. Chem. Commun., 6, 498.
2. Kolthoff and Lingane, Polarograph. Interscience Publishers.
3. Lingane and Loveridge, 1950, J. Am. Chem. Soc., 72, 438.
4. Markowitz and Elving, 1958, Chem. Rev., 58, 1047.
5. Strehlow and Stackelberg, 1950, Z. Electro Chem., 54, 51.
6. Matsuda, 1953, Bull. Chem. Soc., Japan, 26, 342.
7. Kambara, and Tachi, 195_, Ibid, _5, 454.
8. See also, Rummer, 1952, J. Am. Chem. Soc., 74, 3567.
9. Chaturvedi, Doctoral Thesis, Agra University, 1960.
10. Ibid.
11. Brownlie, 1950, J. Roy. Tech. Coll. (Glasgow) 5, 161.
12. King and Cathcart, 1936, J. Am. Chem. Soc., 58, 1639.
13. Cf. ref. 8.
14. Markowitz and Elving, 1959, J. Am. Chem. Soc., 81, 3518.
15. Airey and Smales, 1950, Analyst, 75, 287.
16. Meites and Meites, 1951, J. Am. Chem. Soc., 73, 395.
17. Cf. ref. 7.
18. Macero and Rulfs, 1959, J. Am. Chem. Soc., 81, 2944.
19. Koutecky, 1953, Czech. J. Phys., 2, 50.

CHRONIC LEG ULCERS—A CORRELATIVE STUDY OF HISTO-PATHOLOGY BACTERIOLOGY AND NUTRITIONAL STATUS OF THE INDIVIDUAL

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THE AIM AND OBJECT OF THE WORK

It was increasingly becoming noticeable that cases of chronic leg ulcers are daily attending the various out-patients departments of the S N Medical College and Hospitals, Agra. Review of literature revealed that hardly any work had been done in Northern India on the aetiology & pathology of leg ulcers and its relation to Nutrition. This is what propted us to make a thorough systemic and methodical study of various aetiological factors. Particular attention has been focussed on to assess the role played by the nutritional status of the individual. Awareness that an earliest possible diagnosis is the first and foremost aim in the eradication of cancer necessitated us to perform a routine histological study of biopsy material from persistant ulcers

To be precise, the object of our present work is aimed at —

(1) To elucidate the influence of the nutritional status of the Individual in the causation or on the persistance of leg ulcers.

(2) To isolate the various roganisms from the bacterial flora present in the ulcer

(3) To correlate the histopathological findings with clinical diagnosis.

(4) To assess the incidence of malignancy in such chronic ulcers.

MATERIAL AND METHODS

Materials for the present study were obtained from the Out patients Departments of Dermatology Orthopaedics and Surgery in the S N Hospital, Agra. A group of patients were admitted to the indoor hospitals for further clinical and laboratory investigations and adequate treatment.

Laboratory investigations included blood for Hb %, total erythorcyte count and total and differential leucocyte count. Urine was examined for th presence of sugar and other abnormalities. Serologic tests for syphilis were performed namely V D R L., Kahn test Aldehyde and Chopra tests in suspected cases of Kala-azar or dermal leishmaniasis.

This is an abstract of the thesis approved for the M D Degree in Pathology of the Agra University for the year 1960.

Bacteriologically a direct smear was first examined by staining with Gram's Method, Ziehl Neelsen's stain Leishman's stain and Neisser's stain. Cultures were done both aerobically and anaerobically in suitable media. Isolated organisms were further confirmed by biochemical reactions.

For the assessment of adequacy of nutrition the questionary method as devised by the Nutrition Advisory Committee of Indian Council of Medical Research has been adopted. In deciding adequacy information was obtained on weekly purchase in order to arrive at daily intake. It was proposed that four measures be used to help assessment of adequacy as follows —

(i) Measure which would contain the amount recommended by the Nutrition Advisory Committee

(ii) Measure which would contain ¾ the amount recommended by the Nutrition Advisory Committee

(iii) Measure which would contain ½ the amount recommended by the Nutrition Advisory Committee

(iv) Measure which would contain ¼ the amount recommended by the Nutrition Advisory Committee.

On the above basis, cases were classified into the following four nutritional groups —

Good—Consumption equal to N A. C. recommendation

Fair—Consumption equal to ¾ or above N A. C. recommendation.

Poor—Consumption between ½ to ¾ the N A. C. recommendation.

Very Poor—Consumption equal to ¼ or less than N A. C. recommendation.

After selecting a suitable area at the margin of the ulcer 1 Novocain was injected intradermally and subcutaneously around on area about 1.5 cm in diameter

Observations

Observations were made on the basis of age incidence sex incidence religion occupation mode of onset, site and relation to other associated conditions.

Age Incidence

Out of 45 unselected cases of chronic leg ulcer in general age of the patients varied from 10 yrs to 70 yrs—but however the peak age group was between 20 to 40 yrs—24 4 falling between 20-30 yrs. and 26.6 between 30-40 yrs.

Sex

Of 45 patients 39 were males and 6 females

Occupation

Majority were field workers e g cultivators and other manual workers

Mode of onset

22 patients gave a history of trauma of various modes and 20 patients stated that the onset was either as a boil or as a small bleb. In two cases old burn scars started ulcerating spontaneously—both of which turned out to be epidermoid carcinoma on biopsy. In one case ulcer started as a nodular growth. Histologically all the 5 cases of epidermoid carcinoma were grade I.

Site

This was as follows: Knee Joint 5 cases
Region over the tibea 14 cases
Feet, ankle and toes 26 cases.

Of the later 26, 9 were only on the soles, 7 of which were clinically diagnosed as trophic ulcers, the remaining 2 being diagnosed as actinomycosis.

Different diagnosis of our cases, based on histological changes are chronic non-specific ulcer, chronic non-specific ulcer with atypical or simple epithelial hyperplasia, epidermoid carcinoma, tuberculous ulcer (two being lupus vulgaris), actinomycosis, syphilitic ulcer and ulcerated wart.

In every case bacteriological investigations were carried out both by smear examination (direct microscopy) and by culture on selected media both aerobically and anaerobically.

Table 1

Organisms shown in order of frequency of occurrence

Organisms	No of cases	Percentage
Staphylococcus—Albus 18	7	60 0
Aureus 6		
Citreus 3		
Streptococcus —Faecalis 8	13	28 8
Non-haemolyticus—5	9	20 0
Bacillus Fusiformis	8	17 7
Pseudomonas Pyocyaneus	4	8 8
Proteus —Vulgaris 3		
Morgani 1		
Diphtheroids or Organisms Morphologically identical with K. L. B	4	8 8
Micrococcus Tetragenes	3	6 6
Vincent's Spirochaetes	1	2 2%
Bacillus Subtilis	1	2 2%
Friedlander's Pneumobacillus	1	2 2%
No. organisms grown	9	20 9%

In categorising the patients on the basis of nutritional status according to the questionary method adopted by the Nutrition Advisory Committee of the Indian Council of Medical Research, it was found that majority of the patients fell under the Poor category

Following percentage of different nutritional categories were deduced —

Good	4 4 %
Fair	20%
Poor	68 8%
Very Poor	6 6 /

Discussion

Study of aetiology pathogenesis and pathology of chronic leg ulcers merits interest from several aspects of view From the works of most of the European workers it is seen that subject of their main problem has been centred on the vascular phenomena in the causation of leg ulcers. This is what has been called "Haemodynamics" On the other hand majority of the workers in India and Africa have been dealing with the so called "tropical ulcer" or ulcus tropicum

The term tropical ulcer' though, is being widely used, little has been established as to its cause, and it has resulted in a deversity of opinions. Our present work is an investigation on the leg ulcers, taken in general, to find if any specificity can be linked to it on the basis of pathological changes, bacteriological findings and other factors From our observations it is seen that more than 50 per cent of patients are between the age group 20 to 40. This is in compliance with the findings of Rao et al who stated that no age was exempt though adults formed a majority

Two probable explanations can be brought forward Firstly people from 20 to 40 years of age are more subjected to physical work and hence exposed to trauma. Secondly children of younger age group, though equally exposed to trauma, rate of healing is probably more rapid in children than in adults. Both from occupation and type of ulcer it can be concluded that lesser frequency of such infection in females are due to less exposure to physical and out door work.

On the causation of any kind of leg ulcers occupation obviously plays a great role Firstly infliction of trauma undoubtedly bears a direct relation with occupation. Secondly occupation is a measure to the socio-economic status and hence to the nutritional state of the individual Out of 45 patients 26 are some way or other occupied with physical work.

We find it feasible that nutritional deficiency interferes with the rate of healing and not th t t is a direct cause Specific predilection for the leg is that this part of the body is most liable to infliction of trauma Once the ulceration has started, tissues, famished by malnutrition cannot rejuvenate by natural

process. Another important factor is vitamin A, which is also known as anti infective vitamin and is associated with the metabolism of skin tissue in particular. It was also shown by Thompson that deficiency of Vitamin A and essential fatty acids are directly proportional to the frequency and severity of the ulcers.

Dietary analysis of the present series obviates that diets of most of the patients are particularly low in fat contents and hence in fat-soluble vitamins. Most of the patients being vegetarians, only source of fats and fat soluble vitamin are milk and vegetable oils. Similarly milk is the only source of animal proteins.

Therefore, considering the role of various nutritional factors we are of the opinion that nutritional status of the individuals plays an effective role on the persistance of leg ulcers. This is by in the first instance lowering the general resistance of the body and on the part of the tissues and secondly by depletion of several vitamins, particularly vitamin A and C, which are decidedly associated with the metabolism of the skin tissue. Thus both the vitamin deficiency retards the rate of healing of the ulcers.

Various explanations have been laid down by many workers in the past for the specific predilection of lower leg as the commonest site of ulcers. Whatever may be the mode of onset—two satisfactory and logical arguments can be put forward as regards the site. Firstly this is the part of the body which has the greatest proclivity to be traumatised. Habit or necessity of walking bare footed obviously raises this risk.

Secondly peculiarity of blood supply and effect of gravity cause a longer stasis of venous blood in the part. This produces a local physiological anoxaemia. To these is added the lack of subcutaneous tissue, particularly over the tibia and other bony points of the foot. This second factor causes easy break of already devitalised tissue with slightest provocation by trauma. Thirdly bacteria get an easy access by contamination from soil.

From the present observation it can also be stressed that trauma and primary infection play almost a parallel role in the causation of leg ulcer.

Diagnosis of different ulcers in the present series have been given on the basis of histological changes while a parallel study has been maintained on the bacteriology of every ulcer. Such a study creates a position to try to establish a correlation between the two. It has been seen that in all cases such a correlation cannot always be drawn out. There are certain groups of lesions in which body reacts in some specific tissue changes in certain type of infections while in others there is almost a common pathological change. In our series such infection which showed, as with the findings of other workers, some definite tissue changes are tuberculous ulcer, syphilitic ulcers and actinomycotic ulcer. On

the other hand a wide range of bacterial infections produced a similar picture, which has been termed as Chronic nonspecific ulcer

Important significance of routine biopsy in chronic leg ulcers can once more be emphasised due to fact that 1 in 9 of ulcers has turned out to be cancer in the present study Such a early confirmatory diagnosis is of great prognostic value even on the question of life of the patient. Moreover this necessitates a biopsy study of the regional lymph nodes which is not only of prognostic value but also it decides the mode of surgical treatment to be adopted.

It is a notable finding in the present study that there is no specific tissue changes in those ulcers where fusiform bacilli or organisms morphologically identical with diptheria are present. Literature is full of description and observations on the findings of the so called tropical ulcers both in India and other parts of the tropics Emphasis has been laid down on the presence of fusiform bacilli and Vincent's spirochaetes as the causative factor of tropical ulcers. In the present series, 9 out of 45 cases gave positive results for fusiform bacilli on culture and in 2 other cases, these have been seen only on direct smear examination. But in none of the cases there is any specific type of tissue reaction, so that histologic picture is almost same in all other cases of chronic non-specific ulcers Therefore, we do not find any satisfactory reason why such a specific name *tropical ulcer* should be applied to those cases only where fusiform bacilli and Vincent's spirochaetes are present despite the fact the pathological changes do not differ from ulcers caused by other pyogenic organisms either singly or in combinations. So also with those cases where organisms morphologically identical with K. L. B or diphtheroids are detected. What appears more important is that a routine bacteriological investigation is essential more from the point of view of finding out the nature of the organisms as regards their biological characteristics such as whether they are aerobic or anaerobic. In addition a sensitivity test should be done to avoid unnecessary delay in healing of the wounds for lack of proper selection of drugs.

ROLE OF PLEURAL BIOPSY IN THE DIAGNOSIS AND TREATMENT OF PLEURISY WITH EFFUSION

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Multiple aetiological factors are known to produce pleural effusion but in the absence of the confirmation of the diagnosis by routine clinical and laboratory methods, many of them are labelled as cases of idiopathic pleural effusion Little is known of the changes in the pleura during an attack of exudative pleuritis The knowledge of the procedure that would give a quick diagnosis and at the same time not endanger the patient in any way appeared desirable

DeFrancis, Klosk and Albano introduced in 1955 a procedure of needle biopsy of parietal pleura. Breckler et al (1956) performed needle and open biopsy under local anaesthesia in five patients They found open biopsy superior to the use of Vimsilverman needle. Donohoe et al (1957) also used the technique of open and needle biopsy and in seventy three per cent of cases diagnostically useful material was obtained by needle biopsy and it was of noticeable value in tuberculous effusions.

Heller et al (1956) published the results of 22 biopsies performed on 20 patients and showed caseating granuloma in five cases, neoplasm in four fibrosis in nine and insufficient specimen in two cases. William Weiss (1957) performed needle biopsy in 22 patients and in twelve cases biopsy was compatible with tuberculous pathology Mestitz et al (1957) subjected 116 cases to needle biopsy and in 72 cases a definite diagnosis was established Mestitz et al (1958) performed pleural biopsy by pleural biopsy punch (Abrams 1958) in 200 cases and one hundred and four diagnosis were made by biopsy and only one subsequently proved to be wrong

MATERIAL AND METHODS

50 cases of pleural effusion admitted to the Medical Wards of S N Hospital, Agra were taken up for this study These included 36 males and 14 females. Clinical examination followed by laboratory investigations was done in each case The cases were then subjected to needle biopsy of parietal pleura. Pleural fluid was taken out at the same sitting as pleural biopsy The details of the method include a preliminary examination of the patient at the time of admission and taking down a detailed history of the onset of the symptoms and progression of the disease. Past history was carefully enquired with special

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reference to any history suggestive of tuberculosis or malignancy Similarly family and social history was elicited and any sign and symptom of any help in ascertaining the cause of effusion was noted. Respiratory system was examined in great details with special reference to the presence of pleural disease and effusion and for any diseases of the underlying lung paranchyma.

Laboratory investigations included complete haemogram, bleeding and coagulation times and plasma proteins and albuminglobulin ratio. A routine examination of urine and stool was done. Sputum was examined for the presence of acid fast bacilli and wherever needed it was examined for malignant cells. Mantoux test was performed in different dilutions and a negative Mantoux in 1 100 was considered to be evidence against the effusion to be non-tuberculous This was followed by the radiological examination. Pleural biopsy and paracentasis thoracis was done at the same sitting in every case.

Pleural Biopsy

No special preparation was required before the biopsy Only a mild sedative like gr 1 of luminal was given half an hour before biopsy

Every one of the 50 cases was subjected to needle biopsy of the parietal pleura. In 11 cases biopsies were done on two occasions and in another three cases it was done three times. In all 69 biopsies were done These included 8 cases where pleural biopsies were taken to watch the changes in the pleura during therapy

Technique

A small intra-dermal wheal was raised by injecting novocaine solution in the inter-costal space already selected near the upper border of the lower rib. Local anaesthetic was injected through a 19 gauge needle or a longer one if the subject was obese The whole thickness of the chest wall from the skin down to the parietal pleura was anasthetised 10 to 20 c c. of pleural fluid was aspirated for laboratory examinations. The needle was withdrawn little by little till at a point no more of the fluid came out on suction. The haemostat was clamped at the skin surface on the aspiration needle. The needle was withdrawn and th distance between the haemostat and the point of needle was a measure of the depth of the parietal pleura.

This distance minus 1 Cm approximately was measured on the outer hollow ensheathing of the VimSilverman needle and marked with another haemostat A small nick in the skin was made at the sit of theraco-centeces. The stylus of the biopsy needle was not used. The cuttin prongs were inserted with th ensheathing needle along th tract of the infilterating needle keeping its tip just inside the bevelled sharp edge of the outer ensheathing needle. The Vim-Silverman needle was inserted upto the mark of haemostat which ensured that the tip of th ensheathing needle was well outside the external surface of the parietal pleura. Th inner split needle was advanced to its fullest depth and rotated by 360 degree or more till a

piece of pleura was cut. This was felt by peculiar sensation and sudden lowering of resistance in rotating the inner split needle With one hand holding the outer hollow needle in situ, the inner split needle was gradually withdrawn with the other hand. Pleural tissue was found in the hollow of the legs of the split needle. Pleural tissue was transfered to 10 per cent formal saline The inner split needle was again put in the ensheathing coat, and directed in another direction In this way more than one piece of parietal pleura could be collected at the same sitting The incised wound was sealed and pressure bandage applied.

No important post biopsy care was required except patient was kept in bed for two hours after biopsy

The biopsy pieces were transfered to 10 per cent formal saline They were blocked, sectioned and stained with Haematoxaline and Eosin. Special stains were used wherever indicated.

Observations

The youngest patient was aged four years and the oldest seventy five years Maximum age incidence falls in the age group of 21 to 30 years.

Out of the total number of 69 biopsies performed in 50 cases, adequate tissue was obtained in 63 biopsies. Adequate tissue was thus obtained in 45 cases. The average length of the tissue was about 2 to 3 mm.

No serious complications or untoward side effects were observed in any of the patients during or after performance of pleural biopsy The minor complications met in the present study are given below —

Table 1

Complications in 69 pleural biopsies

Complications	No. of Patients	Percentage
During biopsy		
1 Bleeding from puncture site	1	1 4
2. Sudden acute pain at the site of puncture	1	1 4
3 Liver puncture during biopsy	1	1 4
Post biopsy		
1 Focal sepsis	1	1 4
2. Severe pain at the site of biopsy	1	1 4

One case of mediastinal syndrom had dilated veins on th chest wall and one of these was injured during the introduction of Vim-Silverman needle. Bleeding was immediately controlled, and after waiting for 15 minutes a successful biopsy was performed without any untoward effects

In another case with marked enlargement of the liver and with small pleural effusion a small piece of liver was obtained.

Pathology

The pleural biopsy material adequate for histological examination was available in 15 cases. On the basis of histology of patietal pleura the cases may be grouped as follows —

Tuberculous—12 cases

These showed area of caseation with epitheloid cells, chronic Inflamatory cells including lymphocytes and plasma cells. Typical Langhan's giant cells and variable amount of fibrosis was present in every case. In one case only Tubercle bacilli were found on special staining

Malignant—2 cases

Histological picture of malignant tumour Seminoma was seen in one case and in another case it was typical of Lymphoma

Acute Pleuritis—1 case

Acute inflamatory cells mainly consisting of polymorph cells with little of fibrosis were seen in one case

Non-specific changes—30 cases

These cases showed on histological examination chronic inflamatory cells including lymphocytes and plasma cells. In few of the cases these cells were seen collected in small foci without evidence of much of the fibrosis. In other cases fibrosis was marked feature without much collection of inflamatory cells.

The aetiological diagnosis of pleural effusion on the basis of histology of parietal pleura was therefore certain in 15 out of 45 cases. The remaining 30 cases showed non-specific changes.

On the basis of physical examination and routine laboratory investigations, provisional clinical diagnosis of 50 cases of pleural effusion was made as follows —

Tuberculous—39 cases

Past history of haemoptysis and tuberculous abdomen was found in two cases, while tuberculous glands were present in 12 cases.

Mantoux test was carried out in 27 out of 50 cases. It was positive in all the cases in different dilutions except in one in which it was found to be negative in 1 : 100 dilutions

Effusion was straw coloured in 27 cases, empyema in 8 and hemorrhagic in 4 cases. It was exudate with total cells between 125 to 4000 Cu. mm. and had preponderence of lymphocytes. Total protein content varied from 3.2 to 11 Gms. per cent, and glucose contents estimated in 28 cases ranged between 30 to 106 mgm. per cent

Smear examination of centrifuged specimen of pleural effusion showed tubercle bacilli on special staining in one case only. Infiltration of lung parenchyma was seen in skiagram chest in 5 cases. Sputum was positive for tubercle Bacilli in only one patient. E.S.R. ranged between 14 to 49 mm. after one hour (Wintrobe).

Thus out of 39 cases the diagnosis of tuberculous pleural effusion was certain in 7 cases. The diagnosis of the remaining cases was based on the statistical probability of tuberculous aetiology because of young age and the nature of pleural effusion.

In comparative study of the histology of parietal pleura in these 39 cases, it was found that 10 cases had histology of tuberculous granuloma, while one was proved to be case of acute pleuritis. The remaining 26 cases had non specific inflamation of pleura.

Malignant—11 cases

The definite clinical diagnosis of malignancy was made in 3 cases where malignant cells were found on cell block examination of pleural fluid. Another case presented the symptoms of mediastinal syndrome. Seven other cases were diagnosed clinically malignant effusion because of advanced age and haemorrhagic pleural effusion.

Mantoux test found to be negative only in one case in 1 : 100 dilutions.

Total cells in pleural fluid ranged between 1050 to 4500/ cu-mm. with preponderence of lymphocytes. ESR values ranged between 10 to 60 mm. after one hour.

Values of total proteins were found to be above 3.5 gm. per cent and glucose contents ranged between 30 to 100 mgm. per cent.

Skiagram of the chest was diagnostic only in one case of mediastinal syndrome.

The histology of parietal pleura was diagnostic in only two cases. Non-specific changes were found in four cases. Two cases of this group showed histology of tuberculous pleuritis. In the remaining three cases tissue was insufficient for diagnosis.

Changes in pleura during therapy

Serial pleural biopsies were done in 8 cases, out of these 4 cases were cases of tuberculous pleurisy confirmed by pleural biopsy, 3 cases were of non-specific pleural changes observed histologically and one case was of lymphoma with malignant changes in the pleural tissue.

The most constant changes histologically were in the form of increasing fibrosis and diminution of inflamatory cells. Disruption of tuberculous granuloma due to increasing fibrosis was observed in tuberculous histology of pleura, while in case of lymphoma there was increasing fibrosis and diminution in the number of the tumour cells.

DISCUSSION

The evalution of pleural biopsies has shown that it is simple safe and is no more distressing then a simple pleural aspiration Similar were the observations of Jack D Welsh (1958)

Our modification of Defrances, Klosk and Albano (1955) technique has the advantage of avoiding the re-introduction of biopsy needle for collecting more pieces of parietal pleura.

No pre-operative medication is required as has also been reported earlier by Willium Weiss (1957) nor any special post operative care is needed.

No serious complication were met excepting obtaining a piece of liver in one case without any adverse effect to the patient. Heller et al (1956) described a case in which lung tissue was obtained during the pleural biopsy and small haemo-thorax was noted in another case. Mestitz et al (1957) also reported small haematoma in one case and small pneumo-thorax in another case, neither of which required any treatment.

Adequate tissue for histological diagnosis was obtained in 86.9 per cent of the 69 biopsies performed in the present series, which compares well with Donohoe et al (1957) who got useful material for diagnosis in 73 per cent of cases.

The diagnosis of aetiology of pleural effusion was possible in the biggest series of two hundred cases so far reported by Mestitz et al (1958) Hill et al (1958) obtained 64 per cent diagnostic biopsies when they cultured the pleural biopsy material in addition to the histological study The definite diagnosis in 33.3 per cent of the 45 cases in whom pleural biopsy material was available in our series approximates to the results published by Heller et al (1956) (40.9%), Donohoe et al (1958) (42.2%) and are much better than those reported by Misra et al (1959) (14.2%)

Our failure to give definite diagnosis in 66 7 per cent of cases might be explained on the basis of patchy distribution of tubercles and metastesis of the surface of parietal pleura, marked pleural fibrosis in long standing effusions and anti-tuberculous therapy before admission of the patient in the Hospital. Mestitz et al (1959) in his personal communication has drawn the attention for thicker and serial sections and we have confirmed his suggestion.

The place of routine investigations in these cases require evaluation in view of the claims of pleural biopsy Pleural biopsy gave positive diagnosis of the cause of effusion in 33 3 per cent cases against 8 per cent diagnosed by other investigations.

No reliance can be placed on Matoux test as this test was found to be positive in all the cases of tuberculous, malignant effusions and in cases showing non-specific pathology of pleura. Mestitz et al (1959) did not come across any case which could be tuberculin negative and yet showing tubercular histology of pleura.

The total and differential cell count of pleural fluid gave no indication of the aetiology of the effusion in the present series. Zluneman et al (1957) Sprig gs (1957) Cecil et al (1955) had similar experiences.

The value of glucose content of the pleural fluid was considered valuable by Barber et al (1958) in the differencial diagnosis of the cause of effusion and pleural fluid glucose 20 mgm. or less was highly suggestive of tuberculous effusion while 80 mgm. or more were suggestive of non-tuberculous effusion. The present study has failed to substantiate these observations.

E.S.R. values ranged between 10 to 60 mm. (Wintrobe) in the present series. Normal values were observed in both tuberculous as well as malignant cases. These observations are in agreement with Mestitz et al (1959) and Roper and Warning (1955) who found E. S. R. to be of no help in the differencial diagnosis of pleural effusions.

Radiological infiltration of lung parenchyma was seen in five cases, 4 of which showed tuberculous granuloma and one non-specific histology on pleural biopsy Hill et al (1958) reported 4 positive biopsies of tuberculosis out of 9 cases which showed infiltration of lung on skiagram chest.

The appearence of tubercle bacilli in sputum in one case and in another in centrifuged specimen of pleural fluid confirmed the nature of effusion The histology of pleura in both the cases showed non-specific changes. Negative pleural biopsy therefore does not rule out the tuberculous aetiology of pleural effusion. Jack D Welsh (1958) had also reported cases with positive bacteriology and yet showing a negative pleural biopsy

Malignant cells were found on cell block examination of pleural fluid in 3 cases of malignant effusion, while pleural biopsy was diagnostic of metastetic seminoma in one case only Cell block examination of pleural fluid may therefore, be of considerable help in the diagnosis of malignant pleural effusion.

The serial pleural biopsy during the course of treatment could be used to judge the progress of the case. There is increasing fibrosis with diminuition of chronic inflamatory cells in the parietal pleura in cases of tuberculous pleural effusion and in cases of non- pecific histology of pleura.

Summary and Conclusion

69 pleural biopsies were performed in 50 cases which included 36 males and 14 females ranging in age between 4 to 75 years.

The present technique of pleural biopsy is a modification of the technique used by Defrances et al Different dvantages of the technique have been de scribed. There were no serious complications.

The value of clinical examination, sputum skiagram, chest, Mantoux test and pleural fluid examination have been discussed. Glucose content of the fluid and E. S. R. were found to be of no help in the differencial diagnosis of

DISCUSSION

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STUDIES ON INDIRECT METHODS OF DETERMINING IN THE FIELD PASTURE HERBAGE INTAKES OF GRAZING ANIMALS AND EVALUATION OF THEIR NUTRITIVE VALUES BY MARKER TECHNIQUE

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Though the role of properly managed pastures in maintaining the animal population to give the maximum returns at the lowest cost has always been recognized, the correct assessment of the quality and quantity of pasture herbage in the form they are actually grazed has not been possible due to lack of any reliable method. The progressively changing chemical physical and botanical characteristics of the forage that accompany growth and the tendency of the animals to graze selectively pose additional problems. Recognition of such facts, within recent years led workers in field of Animal husbandry to propose a number of markers or indicators like silica, iron oxide chromic oxide polyethyleneglycol, lignin, chromogen(s) and nitrogen etc which would permit an indirect measurement of the quantity and quality of pasture herbage grazed by the animals. Since the amounts of nutrients ingested by animals during grazing and the extent to which these meet their nutritional requirements is of obvious importance, the present investigations were under taken by the author to study (a) the efficiency of chromic oxide as a marker of dry matter faecal output, (b) the value of faecal nitrogen as an index for estimating intakes by grazing animals, (c) accuracy and value of double indicator technique—using Cr_2O and nitrogen—as substituent of the ordinary conventional digestibility trials, and (d) application of this technique in field trials in the determination of pasture quality and the nutrients ingested by the grazing animals vis-a-vis their requirements

A. *Chromic oxide as external indicator for estimating the faecal production.*

For the determination of faecal output various indigestible indicators added externally to the feed have been suggested but of all chromic oxide has been subjected to a large number of tests and appears to have a high potential value for the estimation of faecal outgo which is a pre-requisite to the measurement of dry matter intake of grazing animals

As a result of an experiment conducted on *hariana* bullocks, fed on sesame cake and wheat *bhoosa* and dosed with 5 g. Cr_2O_3 per day it was observed that percentage recovery of the indicator in faeces varied widely from animal to animal as also from day to day The average recoveries ranged from 101 02

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± 1.20 to 113.11 ± 4.29 per cent on the sixth day and first day respectively. In another experiment but with green feeds, it was observed that the average recoveries for four and seven day periods were 106.89 ± 2.54 and 102.11 ± 2.02 per cent. The results of five subsequent experiments showed that the percentage recoveries ranged from 95.82 ± 1.48 to 98.90 ± 1.33 for hill bullocks and 97.17 ± 0.23 to 101.47 ± 1.28 for buffalo calves.

A study was further made on buffalo calves to find out the excretion behaviour of this indicator and it was observed that on average the Cr_2O_3 concentration (in mg/g dry faeces) goes down steadily from 4.3093 at 7 a.m. to 3.7400 at 4 p. m. after which it rises to 4.7115 mg/g. dry faeces at 7 p.m. Significant differences were found in the excretion of Cr_2O_3 showing that single random faecal sample would be inadeqate for the estimation of chromic oxide upon which depends the determination of faecal output. In another study it was found that in hill bullocks also there was higher excretion of Cr_2O_3 at 7 a. m. than at 4 p. m. When, however the samples collected at these two hours—7 a.m. and 4 p.m.—were composited on an equal wet weight basis it was found that the Cr_2O_3 concentration in such a sample was very close to that in the total collection faeces specimen when 100 per cent recovery of the indicator was assumed. Repetition of the experiments on similar lines on both the classes of animals further corroborate these observations.

A comparison of the measured and the estimated (using Cr_2O_3 as indicator) faecal output values showed that the percentage deviations ranged from -1.75 to $+3.23$ in hill bullocks and from $+1.01$ to $+2.81$ in buffalo calves. Statistical examination also did not show significant differences between the two sets of figures.

Since the Cr_2O_3 excretion pattern in grazing animals was found to be quite similar to that obtained in the stall fed animals, it was considered that under identical dosing, feeding and management conditions the animals may be expected to present a similar excretion pattern of this indicator. Evidently it may also be expected that the concentration of Cr_2O_3 in the faecal grab samples taken at 7 a.m. and 4 p. m. and compounded on an equal wet weight basis would also give an estimate of faecal output in grazing animals also within reasonable limits of accuracy.

B. Faecal nitrogen as an index for estimating intakes by grazing animals.

The indirect measurement of the herbage intaken by animals involves two steps (i) the determination of faecal output and (ii) the determination of feed indigestibility. As shown above Cr_2O_3 may be used for the faecal output determination. The feed indigestibility may be determined by employing either (a) ratio technique or (b) faecal index technique. For the former the indicator should be a natural constituent of the feed and the estimation of the same in feed and faeces both and using the following formula gives the desired value.

Digestibility of

$$\text{herbage (\%)} = \frac{(\text{conc. of indicator in faeces} - \text{conc. of indicator in feed}) \times 100}{\text{Concentration of indicator in faeces}}$$

For the latter the indicator need not necessarily be indigestible and only certain relationsh ps are first established between the indigestibility of herbage and the concentration of that constituent in faeces The latter technique therefore is an improvement over the former since it does not require an assumption of the indigestibility of the plant constituent and requires the analysis of the faeces alone instead of faeces and herbage both as is requ ed in the ratio techniqu.. In either case the herbage intaken by the grazing animals may be estimated by the following relationship

$$\text{Herbage intake (g dry matter/day)} = \frac{\text{faecal output (g dry matter/day)} \times 100}{\text{Indigestibility of dry matter (\%)}}$$

In the present investigations nitrogen was used as the faecal ndex of indigestibility of herbage and the following relationships were worked out for both hill bullocks and buffalo calves combined together

1 D M. intake in kg /day $= 0.128x_1 + 1.41$ ($r = 0.939$ $P < .001$)
2 O M. intake in kg /day $= 0.11\ x_1 + 1.34$ ($r = 0.943$ $P < .001$)
3 D. M digestibility (%) $= 11.42x + 42.52$ ($r = 0.973$ $P < .001$)
4 O M. digestibility (%) $= 11.24x + 45.88$ ($r = 0.976$ $P < .001$)
5 Intake factor for D M. $= 1.055x + 0.945$ ($r = 0.963$ $P < .001$)
6 Intake factor for O M. $= 1.243x + 0.874$ ($r = 0.965$ $P < .001$)

where x_1 = total faecal nitrogen/day
x = % nitrogen in ash free faeces
D M. = dry matter
O M = organic matter
Intake factor = feed/faeces ratio

The results of the excretion pattern studies of this feed constitu nt showed that for the same group whether n stall or under grazing the faecal nitrogen concentration did not differ appreciably from time to time. Further it was observed that a sampling pattern similar to the one followed in the case of Cr_2O_3 (compositing of faecal samples, taken at 7 a. m and 4 p m. on equal wet weight basis) could be adopted here also

Thus employing the grab technique along with the use of two indicators —Cr_2O_3 and nitrogen—the dry matter and organic matter intakes of the stall fed animals were estimated and were not found to be significantly different from those actually measured by the conventional procedure

C. *Studies on digestib lity determination—conventional or double indicator technique*

The conventional method of determining digestibility coefficients of livestock feed is time consuming laborious and expensive. Besides difficulties

are also experienced with female animals in the collection of the uncontaminated faeces.

Under the stall conditions the composition of the herbage offered to the animals may be determined easily by obtaining a representative sample of the feed. The composition of the faeces may be obtained by employing the equal grab technique. Further employing the double indicator technique—Cr_2O_3 for faecal output and nitrogen for indigestibility determination—both the dry matter faecal output and dry matter intakes may be determined. With the knowledge of the above the digestibilities of proximate principles may easily be worked out. Four experiments were carried out as under

(1) *Experiment on hill bullocks fed sarwala grass (Heteropogon contortus) at young stage*

A comparative study of the chemical composition determined by the two procedures—conventional and composite grab sample—showed that with the exception of ether extract, which tended to become significant, the percentage composition in faeces did not differ significantly from each other

The average digestibility coefficients were found to be dry matter 60.73 $\pm$ 1.25 crude protein 54 07 $\pm$ 1 74 ether extract 20 93 $\pm$ 0.31 crude fibre 72 28 $\pm$ 0 71 and nitrogen—free extract 58.95 $\pm$ 1 09 and total digestible nutrients available per 100 kg feed were 58.88 $\pm$ 1 19 by the conventional procedure the respective figures determined by the employment of double indicator technique were 59 44 $\pm$ 0 62, 54 16 $\pm$ 0.99 12 99 $\pm$ 0.57 72.23 $\pm$ 1 03, 56.48 $\pm$ 0 67 and 59 80 $\pm$ 1 70.

(2) *Experiment on buffalo calves fed on sarwala grass at pre flowering stage*

Here it was observed that with the exception of crude fibre and nitrogen-free extract the faecal composition (per cent dry basis) determined by the grab technique did not differ significantly from that determined by conventional method.

The average digestibility coefficients were dry matter 55.82 $\pm$ 0 86, crude protein 37 17 $\pm$ 0 86, ether extract 26.25 $\pm$ 2 17 crude fibre 67.51 $\pm$ 1 45, nitrogen free extract 57.97 $\pm$ 0 97 and T D N per 100 kg feed 51.90 $\pm$ 0.79 by the conventional procedure The respective figures determined by double indicator technique were 55.09 $\pm$ 0 34 34 01 $\pm$ 1 18 25 14 $\pm$ 3 19 66.35 $\pm$ 0 61 53 63 $\pm$ 0 89 and 54.20 $\pm$ 0 30

(3) *Experiment on buffalo calves fed on Russian rye grass (Elymus junceus) at pre flowering stage*

Here too it was observed that with the exception of ether extract which tended to become significant the chemical composition of faeces determined by grab technique did not differ significantly from that determined by conventional method.

The average digestibility coefficients were dry matter 74 76 ± 1 11 crude protein 62.18 ± 1 79 ether extract 72.40 ± 0 85 crude fibre 82.84 ± 1 09 and N.F E. 76.69 ± 1.39 and T D.N /100 kg.feed 74 42 ± 0 74 by the conventional procedure. The respective figures determined by double indicator technique were 73.42 ± 1 11 60.02 ± 0.41 64 99 ± 2.49 81 33 ± 0 73 75.27 ± 1 83 72 66 ± 1.04

(4) *Experiment on hill bullocks and buffalo calves fed on banchari grass (So ghum halepense) at pre-flowering stage.*

It was observed that with the exception of nitrogen—free extract in the buffalo calves group which was found to differ significantly the faecal percentage composition with respect to other constituents did not differ significantly in either group by the two methods.

The average digestibility coefficients determined by conventional procedure with respect to dry matter crude protein ether extract, crude fibre and N F E. were respectively 59 78 ± 0.57 61 45 ± 0 93 46.80 ± 2.33 74 13 ± 1 58 and 57 62 ± 0.24 for the bullocks and 59.86 ± 1 02 61 44 ± 0.88 46.37 ± 2.11 72.09 ± 1 03 and 57.59 ± 1.02 for buffalo group The T D N / 100 kg feed was 59.38 ± 0.56 and 58.72 ± 0.89 respectively for the two classes of animals.

The average digestibility coefficients determined by double indicator technique with respect to dry matter crude protein, ether extract, crude fibre N F E. and T D N./100 kg feed were found to be 60.23 ± 0 33 61 14 ± 0.30, 49.57 ± 1 37 74.25 ± 0 99 58.13 ± 0.43 and 59 71 ± 0.36 for bullocks and 60.22 ± 0.36, 60 83 ± 0.34 42.25 ± 2.36 72.24 ± 1 07 59 04 ± 0.37 and 59.36 ± 0.37 for buffalo calves.

The statistical examination of the data showed that with the exception of ether extract n two experiments and nitrogen-free extract in one experiment the digestibility coefficients of proximate principles as also T D N available /100 kg feed determined by the two procedures were not significantly d fferent from each other

It, therefore appeared that the double ind cator technique using ch omic oxide as indicator of faecal output and faecal nitrogen as index of feed indigestibility in the determination of digestibilities of various nutrients under stall conditions provided a handy tool in conducting digestibility trials wi h economy and within reasonable limits of accuracy

D *Determination of pasture quality and the nutrients ingested by grazing animals vis-a-vis their requirements*

At present time the most practicable measures of total nutriti e value of pasture herbage are the digestibility and rate of consumption of dry matter by grazing animals. Though under practical feeding conditions the measure of feed value in terms of its T D N content has been considered to be more

valuable, its determination requires the composition of feed with respect to crude protein ether extract, and total carbohydrates as also their respective digestibilities, both of which are difficult to determine under grazing condition. In the present investigations, therefore, recourse has been taken to determine indirectly the pasture quality as grazed by the animals on the basis of the data obtained by the indoor digestibility trials run side by side. Three experiments were conducted as follows

(1) *Experiment on hill bullocks grazed on sarwala grass pasture at young stage.*

From the knowledge of the chemical composition of the herbage as grazed by the individual animals determined indirectly taking the faecal composition of the stall fed animals as the basis the digestion coefficients with respect to the dry matter crude protein ether extract, crude fibre, N F E. and T D.N. available /100 kg feed were found to be 65 41 ± 0.40 59 60 ± 0.47 50.43 ± 0 84 75.58 ± 0.28 65 49 ± 0 62 and 62.23 ± 0 18 Chromic oxide and nitrogen were used as the two indicators as usual.

(2) *Experiment on buffalo calves grazed on sarwala grass pasture at pre-flowering stage*

The composition of herbage grazed by the animals was determined by the indirect procedure for the individual animals and digestibilities worked out. On an average, the digestibility coefficients with respect to dry matter crude protein ether extract, crude fibre, N F E. and T D N./100 kg feed were shown to be 60 95 ± 0 85 44 56 ± 1.21 34 77 ± 1.35 66 79 ± 0 72, 63.93 ± 1 00 and 57 45 ± 0.22

(3) *Experiment on buffalo calves grazed on Russian rye grass pasture at pre-flowering stage.*

Working on similar lines the digestibility coefficients on an average with respect to dry matter crude protein ether extract crude fibre, N F E. and T D N /100 kg feed were found to be 76 76 ± 0 85 65 20 ± 1.29 74 61 ± 0.94 84 18 ± 0.58, 79 04 ± 0 94 and 73 01 ± 2.04 respectively

Possible factors which influence animals to graze selectively have been discussed. In the present investigations it was observed that out of the three experiments conducted in the field the differences in the per cent nitrogen in faeces of the two groups—stall fed and grazing—were found to be statistically significant in the first two experiments (one on hill bullocks fed sarwala grass at young stage and the other on buffalo calves fed on *sarwala* grass at pre-flowering stage) while in the third experiment (buffalo calves fed on Russian rye grass) these were not found to be significantly different though the percentage nitrogen in the grazing group animals was still higher than the stall fed animals.

Another evidence to prove the selectivity by the grazing animals has been put forward by considering the herbage composition consumed by the two sets of animals In the first experiment hill bullocks selected herbage

containing 27.86 per cent more of crude protein, 51 11 per cent more of ether extract and 10 84 per cent less of crude fibre from *sarwala* grass than consumed by their counterparts in the stalls, Similarly buffalo calves in the second experiment selected herbage containing 46.10 per cent more of crude protein, 24 17 per cent more of ether extract and 14 15 per cent less of crude fibre from *sarwala* grass at pre-flowering stage than consumed by the stall fed animals. In the third experiment, however buffalo calves selected portions of Russian rye grass containing only 4 44 per cent more crude protein, 73 71 per cent more ether extract and 17 19 per cent less crude fibre than consumed by the stall fed animals.

Further it was observed that, in general, the digestibility was greater for all nutrients of grazed herbage which contained more protein and ether extract but less of crude fibre than fo those containing less of crude protein and ether extract but high crude fibre. Thus the digestibility coefficients of all the nutrients in the grazed herbage were, in general highest in the case of Russian rye grass and lowest in the case of *sarwala* grass both studied at their pre-flowering stages of growth.

With a view to assess the pasture quality to the extent they could satisfy the requirements of the adult and growing animals, the amounts of digestible protein and total digestible nutrients needed for them were computed and compared with those consumed by the grazing animals as determined by the double indicator technique

It was observed that *sarwala* grass pasture at young stage provided more than sufficient digestible protein as well as total digestible nutrients to grazing bullocks. *Sarwala* grass at pre-flowering stage, on the other hand provided about 0.28 kg less of digestible protein and 0.58 to 0 71 kg less of T D N to buffalo calves than recommended for such animals having a mature body weight of 1000 lbs. It is suggested that the deficit should be made good by supplementation with protein and energy rich concentrates. Russian rye grass at pre flowering stage also provided from 0.06 at 0 17 kg less of digestible protein to buffalo calves and must be supplemented with cake.

STUDIES ON THE ELECTROPHORESIS OF SOME LYOPHOBIC SOLS*

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The possibility of directly observing the motion of the ions, first investigated by Lodge was theoretically developed by Kohlrausch & Weber. According to Kohlrausch the relation between the concentration and transference number of two electrolytes A & B forming a stable and sharp boundary is given by the relation $T/C = T/C_a$, where T and T are transference numbers and C and C_a the respective concentrations of the leading and indicator ions. Later on extensive work was done to advance the theoretical concepts and improve upon the experimental technique. Whetham, Masson, Dennison & Steele, Abegg, Gauss, McInnes, Longsworth, Mukherjee, Price & Lewis, Henry and Brittain and their co-workers made important contributions in this field. Their observations established the fact that dependable values of electrophoretic velocity are only obtained when we can choose two solutions AR & BR containing a common ion, such that one ion (A) may lead the other (B) called the indicator ion in its velocity. They also recognised the existence of a restoring effect which partly overcomes the disturbance in the boundary caused by diffusion.

Mukherjee was first to improve upon the simple Burtons tube by introducing side tubes attached to the main U tube for the measurement of the potential across the boundary itself. Price and Lewis placed the electrodes at a considerable distance from the main U tube to avoid the errors due to electrolysis products. The most recent improvement in the technique is due to Thellius who introduced two large electrodes vessels which were attached to a U-tube of rectangular cross section and detachable parts to serve as the Burtons's tube. In this way the tube of migrating boundary was kept undisturbed by the products of electrolysis.

Improvements in the applied electric field were first introduced by Price & Lewis who maintained a constant potential across the main U tube by means of a manually adjustable potentiometer circuit. A photo-cum-mechanical current regulating device was used by McInnes for the determination of transference numbers of ions under constant current conditions. I have been able by means of a technique based on electronic valve circuits, to minimise the changes in potential gradient caused by polarisation and electrolysis.

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In order to study the electrophoretic velocity under constant voltage VR 105 with necessary accessories in the circuit has been used. Study of electrophoresis under constant current conditions were performed by employing a sharp cut off pentode valve 6 SJ7GT whose plate current was independent of its plate voltage according to the characteristic data of the valve. A full unit of the circuit diagram has been described and explained in chapter II of the thesis. The potentio voltmeter system used by Price and Lewis was not free from error due to polarisation during the interval of adjusting the system to a null point. I have measured the potential difference across the main U-tube, free of the aforementioned error with high internal resistance vacuum tube voltmeter in place of the low resistance galvenometer in the potentiometer circuit

With such arrangements made possible by electronic control, I have attempted to study the conditions under which the ζ-potential should be determined

The first series of the experiments was conducted under unstabilised field conditions where the observations were full of uncertain irregularities. An electronic voltage regulator (described in Chapter II of this thesis) consisting of a VR 105 was then employed. The reproducible and regular shape of the velocity time curves indicated that the electrophoretic velocity and the distance moved by the particles to form a sharp and compact boundary varied with the potential gradient. The effect of current reversal showed a marked change in the electrophoretic behaviour of the colloidal particles, their velocity was reduced and even a sharp boundary was not formed on reversing the current once or twice, which depended upon the nature of the Sol. By changing the concentration of the supernatant liquid the colour of the advancing Sol column $Fe(OH)_3$ Sol changed from yellow→orange→red→deep red as the concentration of the supernatant liquid was gradually increased The different colours of the advancing Sol column are possibly due to the adjustment of the concentration of the indicator ion according to the requirement of Kohlrausch relation T /C =T /C

Studies under constant current are logically preferable because of the simpler variation of the driving potential (V=I×R) across each of the small liquid elements (two of which contain the boundary under investigation) This potential across any element has been discussed theoretically on the basis of Ohms Law to depend only upon the change in the resistance of that very element under the conditions of constant current (page 64 Chapter V)

My studies on electrophoretic velocity under constant current are divided under the following heads

(1) Influence of various equi-conducting liquids as supernatant liquids.
(2) Effect of different concentrations of a suitable supernatant liquid.
(3) Effect of dialysis of the Sols and
(4) Effect of dilution of the Sols.

The sharpness and diffusion of the colloid boundaries with different equi conducting supernatant liquids could be explained on the basis of Kohlrausch Weber theory For example, the observed mobility of the Fe(ic) micelle was 44 while that of the Li ions was 39 thus the descending boundary of th $Fe(OH)_3$ —LiCl Solution (of same conductivity) system should be sharp and this has actually been observed. Yet in a few cases just the reverse results have been obtained *e g* the sharp ascending boundary of the Sb_2S_3-equiconducting acetic acid system, where the velocity of the Sb_2S_3 micelle was 51 and of CH_3COO was 42.

Changes in the aggregation of the particles during electrophoresis were observed in the case of Au Sol and the simultaneously diffuse ascending and descending boundaries of Cr $(OH)_3$ suggest some role played by the colloid ele ctrolyte interaction under the tension of an electric field.

According to the Kohlrausch Weber theory the leading and the indicator ion should move with the same velocity when the sharpness of boundary has taken place but my experimentally observed data does not confirm this aspect in all cases.

Under constant current the potential across the subsidiary electrodes, measured by means of the potentio V T V M circuit shows a conti nuous change in the resistance of the system. Such a change may be the out come of the colloid-electrolyte interaction like desorption or base exchange pheno-menon under the tension of the electric field.

The effect of variation of the concentration of the supernatant liquid on the boundaries was similar for the positive and negative Sols. Their sharpness, diffusion and colour excepting few cases could be explained on the basis of Ko-hlrausch Weber theory and variation of density of the layers of electrolyte a-djacent to the moving boundary

The study of the effect of dialysis of the Sols on their electrophoretic mobi-lity served as a means of observing indirectly the influence of th ionic conce ntration on th electrophoreti velocity Evidence of the appreciable influence of the ionic strength on the electrophoretic velocity of both positive and negative Sols has been obtained The fact that the electrophoretic velocity tended to increase upto a certain stage of dialysis is explained by the expression $U = \frac{E\sigma}{\eta}\sqrt{\frac{1000\,DRT}{8\pi N^2 e^2 \Sigma CZ^2}}$ which has been derived from the Smoluchowski, Henry and Debye and Huckels equations. From the above expression it is evident that the electrophoretic velocity U is proportional to $\frac{1}{\sqrt{\Sigma CZ2}}$ assuming the other factors remain constant. The observed increase in the electrophoretic velocity on dialysis was therefore due to the fall in the ionic strength of the system. The slight decrease in the electrophoretic velocity after the maxima has been explained by assuming that the thermodynamic

potential of the Sol is changed due to the variation in the activity of the potential determining ions at the surface of the colloid particles (vide sketch No.3 Chapter IX p 106)

The behaviour of Sols with respect to their electrophoretic velocities on dilution has been found to be similar as reported by Mukherjee in the case of $Fe(OH)_3$ Sol, although the technique adopted by me was not identical with his. The change in the ionic strength due to dilution and its consequent effect on the ξ-potential seems to be responsible for the variation of the electrophoretic velocity on dilution The deviation of the behaviour of the As_2S_3 Sol where the electrophoretic mobility decreases after a maxima is attributed to the change in the thermodynamic potential due to the less stable and hydrolysable nature of the As_2S_3 particles (vide Chapter X, p. 113 fig 4 and 5)

Previous authors had given many suggestions for improvement of the techniques to arrive at the correct results. My experience suggests that much improvement may be possible by the growing knowledge of electronic and its application This kind of application may be considered as the first adventure on the study of electrophoresis undertaken by me May my attempts to regulate the conditions of the electric field lay the foundation of further studies of this problem with greater refinements and accuracy by electronic device. The variations in the values of ξ-potential are reported to be as wide as 300%. Such discrepancies might be partly due to the different techniques of measuring the boundary movement and partly to the conditions of the field which does not seem to have been adequately controlled

In spite of the few discrepencies from the Kohlrausch Weber theory observed by me in certain cases, the ingenuity of the relation $T_a/C = T/C_a$ for the sharpness of stable boundary is admittedly a remarkable contribution. The developments of the problem both from theoretical as well as practical aspects by Mukherjee and Henry and Brittain were further achievements to reveal the complexities of the phenomenon of electrophoresis. Keeping in view the critical observations of these great pioneers in view I have extended the studies to several other Sols by the new technique based on electronic device.

The observations discussed in the thesis are fairly suggestive of the conditions which should be maintained in the system for electrophoretic measurements for more correct evaluation of the ξ-potential.

The conclusions derived from my investigations are as follows —

(1) The Sols should be prepared by the same method under identical conditions of concentration mixing and temperature

(2) The same degree of purity dialysis should be attained.

(3) Current should be made constant in the Burtons U tube supplemented by the Tiselius Tubes for eliminating electrolysis products.

(4) Use of a suitable equiconducting electrolyte as supernatant liquid.

(5) Constant temperature to be maintained during electrophoresis.

(6) The ζ-potential should be determined from the measurements in the descending boundary and it should be necessarily expressed as ζ-descending or ζ-ascending along with the name of the electrolyte used as supernatant liquid.

(7) The value of ζ-potential should be calculated when the sharp boundary moves with a constant velocity. The values in the earlier or later stages as depicted by the boundary velocity—Time curves should not be taken as reliable.

(8) Convection phenomenon should be guarded and not allowed to take place.

The conditions (1) and (2) are difficult to be warranted but a close approach towards them will, in my opinion considerably narrow down the wide variations in the values of ζ-potential obtained by different workers.

KINETICS OF THE REDUCTION OF MERCURIC CHLORIDE BY ORGANIC ACIDS INDUCED BY CHEMICAL INDUCTORS

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The reduction of mercuric chloride to the mercurous state by organic acids—oxalic and tartaric—induced by potassium persulphate has been studied from the viewpoint of kinetics and a tentative mechanism for it has been suggested on the basis of the formation of free radicals SO_4^- and CO_2^-

The reduction of mercuric chloride by oxalic acid induced by potassium persulphate, having been studied at various temperatures and concentrations of the reactants is found to be of a zonal character The five distinct zones as exhibited during the course of the reaction are (i) Period of Induction (ii) normal reaction of zero-order (iii) auto-catalysis, (iv) auto-inhibition, and (v) reversible reaction. These zones are found to be effected by temperature and concentration of each reactant. Increase of temperature increases the velocity of overall reaction the period of induction as well as of normal velocity decrease while auto-catalysis is shifted towards earlier intervals Both, the period of auto-inhibition and stage of reversibility set in earlier The total amount of reduction of mercuric chloride is proportional to the concentration of each of the reactants upto a certain concentration depending upon the concentration of the other two reactants. When the concentration of oxalic acid exceeds twice the concentration of the persulphate or the concentration of persulphate exceeds that of the acid thus reduction suffers auto-inhibition. With mercuric chloride concentration the reduction is found to be linear

Carbon dioxide and nitrogen gases, glass surface and sodium acetate are found to accelerate the reduction while H^+ & Cl^- ions and oxygen inhibit it. A tentative mathematical expression (given below) derived on the basis of the chain mechanism enveloping the various facets of the reaction, is found to account for the zonal character of the reaction as well as for th various other observations

$$\frac{d\,c_{HgCl}}{dt} = k \frac{c_{S_2O} \cdots c_{C_2O} \cdots c_{Hg^{++}}}{c_{O_2}\left\{1+k\,\frac{c_{S_2O}}{c_{O}}\right\}}$$

The reduction of mercuric chloride by tartaric cid induced by potassium persulphate has also been investigated from the same aspects as that for the preceding acid. It has been observed that there is no period of induction

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in this case and that the order of the overall reaction under the conditions of my experiments is two With decrease in temperature and concentration of tartaric acid the values of second order velocity constant are found to decrease. Increase of temperature favours the progress of the reaction by second order The total reduction of mercuric chloride in a fixed interval is found to increase with increasing concentrations of tartaric acid, mercuric chloride and potassium persulphate. Various acids, potassium chloride and oxygen act as inhibitors while salts of these acids have no effect on the reduction of mercuric chloride. Carbon dioxide and nitrogen gases, glass surface and sodium thiosulphate catalyse the reduction the effect of sodium thiosulphate being more pronounced The same mathematical expression on substituting tartarate ion in place of oxalate ion accounts for its various features suggesting that a similar chain mechanism is involved in the reduction of mercuric chloride in this case as well

From the comparative angle it may be observed that the reduction of mercuric chloride by oxalic acid is much faster than in the case with tartaric acid In the former case there is a distinct periodicity in the reaction while in the latter no such periodicity is observed e g period of induction and reversibility are not at all observable. The second order velocity constant shows gradual auto-inhibition in the initial stage and then becomes appreciably constant. In the case of tartaric acid the reduction of mercuric chloride is very slow and only at higher concentrations of the acid a measurable velocity is obtained. It was, therefore, necessary to increase the concentration of tartaric acid as it was found that the reduction of mercuric chloride did not proceed with a measurable speed, if the concentration of it was kept the same as that of oxalic acid under identical conditions of temperature and concentrations of inductor potassium persulphate and acceptor mercuric chloride. Another contrasting feature of the reduction of mercuric chloride by tartaric acid was that on increasing the concentration of the inductor a slow and gradual increase in the total reduction was observed as compared to the decrease in the reduction at high concentrations of potassium persulphate in the case of oxalic acid.

As regards the points of similarity it was conceived possible to explain the various features of the reduction of mercuric chloride by tartaric acid by the same mathematical expression obtained for the reduction of mercuric chloride by oxalic acid and it is my confirmed view that similar chain mechanisms leading to a similar mathematical expression is likely to explain the reduction of mercuric chloride by all organic acids induced by potassium persulphate.

Though it is very difficult to formulate a mathematical expression for the velocity constant of the reduction of mercuric chloride taking into consideration all the complexities of the reaction yet I have endeavoured to

include them in the expression for the reaction rate (given above) While I do not claim the final word on the mechanism of the reduction of mercuric chloride the fact remains that this kinetic expression derived on its basis explains the characteristics and the various features of the reaction in a striking manner



THE CHEMICAL INVESTIGATION ON THE STRUCTURE OF ACACIA SUNDRA GUM AND OTHER PLANT GUMS*

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Amongst the gums obtained from the trees of the species of Acacia genus, the studies on the structure of gum arabic have been most extensive Apart from gum arabic the other gums that have been studied are Acacia mollissima, A. pycnantha, A. cyanophylla and A. karroo Of the gums obtained from Indian species only A. catechu appears to have been investigated, though India abounds in such gum producing trees. The present investigation deals with the structure of A. sundra gum and it was of interest to find out what relation it bears to other gums of the Acacia genus The gum was obtained from the Forest Utilisation Officer Poona.

The gum was in the form of clear nodules of yellowish orange colour and was practically free from dirt and bark. It was purified by precipitating its filtered, acidified aqueous solution repeatedly with ethanol. The product was in the form of a light buff-coloured powder

The gum is freely soluble in water and caustic soda solution, forming light yellow opalescent solutions which are very sticky The gum does not reduce Fehling's solution. On analysis, nitrogen, sulphur and halogens are absent pentosans, 23.3% pentoses, 26.5% (calculated on the basis of pentosans) and furfural, 13 8%

Graded Hydrolysis—An aqueous solution of the gum was sufficiently acidic to undergo slow autohydrolysis when the solution was heated on a water bath. The hydrolysis was complete after 80 hr heating, as indicated by iodometric titration. The hydrolysed solution, after neutralisation and concentration, was extracted with methanol The methanolic extract was concentrated to a syrup. A paper chromatogram of the syrup using butanol-water-ethanol (40 19 11) solvent indicated the presence of rhamnose (strong spot) arabinose (strong spot) galactose (faint spot) and xylose (trace) The syrup was resolved into its components by cellulose column chromatography L-Rhamnose, L-arabinose and D-galactose were obtained, which were identified by their melting points, specific rotations and suitable derivatives.

The barium salt of the degraded gum obtained by the methanolic extraction of the autohydrolysed solution, was subjected to further hydrolysis with 0 1N sulphuric acid (35 hr) The methanolic extract at this stage showed th

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presence of D-galactose (main component) and L-arabinose (small proportion). The *methanol-insoluble barium salt of the aldobiouronic acid was hydrolysed* with N sulphuric acid. Its methanolic extract showed the presence of D-galactose only. The mehanol-insoluble barium salt was treated with calculated amount of sulphuric acid. Its paper chromatogram indicated the presence of glucuronic acid (strong spot) and glucurone (faint spot). It gave a negative basic lead acetate test for galacturonic acid. The aldobiouronic acid was reduced with lithium aluminium hydride and then hydrolysed. The hydrolysate on a paper chromatogram gave spots of glucose and galactose, proving conclusively that the uronic acid component of the aldobiouronic acid is glucuronic acid.

Identification of the Aldobiouronic Acid—The barium salt of the aldobiouronic acid was converted into the free acid by removing barium with Amberlite IR 120 (H). On a paper chromatogram using butanol-acetic acid-water (4:1:5, upper layer) solvent, and p-anisidine phosphate spray reagent it showed traces of glucurone, glucuronic acid and galactose which were obviously present as impurities in the barium salt and two components near the origin—one a strong and the other a faint spot. Thus, the aldobiouronic acid is a mixture of two components, a main component and the other in a small proportion. The barium salt of the aldobiouronic acid was methylated with dimethyl sulphate and with Purdie's reagent and fractionally distilled. The fraction distilling over at 210°-40°/0.1 mm. (bath temperature) (methoxyl, 48.0%) was again again methylated with Purdie's reagent and distilled. No increase in methoxyl content was noted. Methanolysis followed by acid hydrolysis of the methylated aldobiouronic acid gives two products (i) a methylated galactose (methoxyl 40.8%, tri-O-methyl galactose requires 41.9 methoxyl) and (ii) methylated uronic acid. The methylated galactose forms an aniline derivative which is 2,3,4-tri-O-methyl N-phenyl D-galactosylamine which proves the sugar to be 2,3,4-tri-O-methyl D-Galactose and also shows that the methylated galactose is linked through its C_6 atom to the methylated glucuronic acid part. The latter was oxidised with bromine, esterified and distilled. It was identified as the methyl ester of 2,3,4-tri-O-methyl-D-glucaric 1,5-lactone. It shows that the uronic acid is linked to the galactose residue through C_1 atom. These observations together with the specific rotation $[\alpha]D^{32} = -19.6°$ of the methylated aldobiouronic acid establish the structure of the aldobiouronic acid as 6-O-β-D-glucuronopyranosyl D- galactose. The same aldobiouronic acid is present in gums obtained from the other species of the genus Acacia e.g. A. verek, A. mollissima, A. pycnantha, A. karroo and A. cyanophylla.

Quantitative Hydrolysis of the Gum—The gum was hydrolysed with 2 N sulphuric acid. The component sugars of the syrup obtained after neutralisation and concentration of the hydrolysate were separated (paper) chromatographically and the individual sugars eluted from the paper strips with water oxidised with sodium metaperiodate and the liberated formic acid titrated with standard alkali. Galactose, arabinose and rhamnose were found to be present

in the ratio of 3:2 1 This ratio agrees fairly well with the one found in the case of A. verek.

Periodate Oxidation of the Gum—When the gum was oxidised with periodate 1.6 moles of formic acid was produced per equivalent weight of the gum, and for each equivalent weight 6.9 moles of periodate was consumed Chromatographic analysis of the periodate-oxidised gum, after hydrolysis, showed that certain of the galactose units and some arabinose units also had survived periodate oxidation. This evidence demonstrates that the gum may be highly branched or may contain a proportion of 1→3 glycosidic linkages, or both and that those galactose and arabinose residues in which branching occurs or which are 1→3 glycosidically linked are not affected during periodate oxidation.

The Structure of the Degraded Gum—The degraded gum forms the basic nucleus of the whole gum during autohydrolysis the labile sugar residues which are attached glycosidically to the main structure are removed and the degraded gum is obtained. The degraded gum is reducing in nature and consists of a repeating unit of 10 D-galactose residues and 4 D-glucuronic acid residues. It was methylated with dimethyl sulphate and with the Purdie's reagent. The fully methylated polysaccharide on methanolysis gave methyl glycosides of methylated D-galactoses and methyl glycoside of methylated D-glucuronic ester This mixture was saponified with barium hydroxide to convert the uronic ester into the barium salt of the uronic acid. Glycosides of methylated D-galactoses were separated from the barium salt by extracting the dried mixture with ether The two fractions were then separately hydrolysed to get the corresponding mixture of methylated D-galactoses and methylated D-glucuronic acid. The latter was 2,3 4-tri-O-methyl-D-glucuronic acid, identified by its RG value (relative to 2,3,4 6-tetra-O-methyl-D-glucose) methoxyl content and conversion into the characteristic crystalline methyl 2,3,4-tri-O-methyl D-glucarat 1,5-lactone Its molar ratio to the total amount of methylated D-galactoses was 2.5 calculated on the basis of the ratio of its weight obtained to the weight of methylated D-galactoses. Mixture of the neutral sugars was separated quantitatively on filter paper sheets by chromatography when three sugars were obtained, molecular proportions of which were calculated from their respective weights recovered 2 3 4-tri-O-methyl D-galactose (6 moles) 2,4 6-tri-O-methyl D-galactose (1 mole) and 2,4-di-O-methyl D-galactose (3 moles) The structures of these were proved by their RG values, demethylation studies, determination of methoxyl content and preparation of aniline derivatives. Melting points of the aniline derivatives and also the RG values of 2,4-and 4-6 di-O-methyl D-galactose are almost the same. The fraction obtained from the methylated degraded A. sundra gum was confirmed to be a 2,4-di-O-methyl-derivative by periodate oxidation when formaldehyde was evolved. This showed the presence of a free primary alcoholic group in the molecule which could be t C_6 only

From the above results it is apparent that the galactose units in the degraded gum which give rise to 2 4-di-O-methyl D-galactose are linked through C_1, C_3 and C_6 and branching occurs at these galactose units, the one which is linked through C_1 and C_3 gives rise to 2 4 6-tri-O-methyl D-galactose and those which are linked through C_1 and C_6 give rise to 2,3,4-tri-O-methyl-D-galactose and the fully methylated D-glucuronic acid units through C_1 each to the main structure and form the ends. On the basis of these considerations a structure has been suggested for the degraded gum.

SYNTHESIS AND REACTIONS OF BROMONITROSO AND BROMOISONITROSO KETONES*

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U P Agricultural University P O Pant Nagar Naini Tal.

In this thesis the author has studied the preparation and properties of some nitroso and isonitroso ketones. In these compounds there are two triad systems superimposed upon one another which give rise to a pentad system of the type found in 2-formyl ketones. A comparison has been made between the pentad system found in 2 formyl ketones with that found in nitroso and isonitroso ketones. The author has prepared these compounds by the action of amyl nitrite on ketones in presence of sodium dust or sodium ethylate in dry ether or by the action of other alkyl nitrites in presence of concentrated hydrochloric acid or dry hydrochloric acid gas in dry ether. In this way several nitroso and isonitroso ketones, have been prepared by nitrosation of ketones.

Nitrosation like formylation can be used to study the carbanion formation in these compounds. In the case of open chain ketones it is found that the action of alkyl nitrite on the two isomeric derivatives gives only one product and the structure of this can be arrived at by studying the products obtained after oxidative fission by potassium dichromate. In the case of aromatic ketones the nitrosation takes place at the methyl group while in the case of cyclic ketones the nitrosation takes place at th methylene group. A comparison of the properties between the pentad system found in these compounds and that found in 2-formyl ketones reveals many similarities.

The author has also studies ∝-bromo-∝-nitroso and ∝ bromo-∝-isonitroso ketones and has compared them with the corresponding chloro compounds. The compounds were prepared by two different routes viz () by bromination of isonitroso ketones and (ii) by nitrosation of 2-bromo ketones. The bromination of ison troso ketones was carried out directly or through sodium salt or through copper complex. Direct bromination of free isonitroso ketones in different solvents such as pyridine, ether chloroform, alcohol, aceticacid and water was unsuccessful and i was found that the free bromine oxidises the f ee isonitroso ketones into corresponding acid. Similar results were found when bromination of sodium salts of free isonitroso ketones was carried out. Bromination by N-bromosuccinamide was also unsuccessful. Bromination of copper complexes however were found to give encouraging results.

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*This is an abstract of the thesis submitted and approved for the Ph.D degree of the Agra University for the year 1960

The nitrosation of bromo ketones was carried out by alkyl nitrite in presence of hydrogen chloride. From the cases studied by the author it appears that on nitrosation in aromatic ketones the group >NOH would enter in the side chain. In case of 2 bromo aliphatic ketones and 2 bromo mixed ketones the group >NOH is taken up more rapidly when adjacent groups present at >C=O are the same. Further the group >NOH attaches itself to that adjacent atom (in reference to >C=O) which does not carry bromine. In the case of *bromo benzoyl acetone* and *bromo menthone gummy products containing* both nitrogen and bromine were obtained. The products were very lachrymatory and the author could not carry out the study for enough to characterise them. In the case of bromo camphor though nitrosation was tried in several solvents and nitrosation was also tried even in presence of acids, the attempts were unsuccessful. This is probably due to the fact that in case of bromo camphor the active hydrogen of camphor is already replaced by bromine and unless the migration of bromine takes place nitrosation would not be possible. Apparently no such migration takes place under the conditions in which nitrosation was tried by the author

The compounds were characterised by a study of their analytical data and oxidative fusion products. The action of sodium sodium hydroxide ammonia, ferric chloride, Fehling's solution, pyridine, potassium iodide hydroxylamine hydrochloride, sulphuric acid and aniline on these compounds has been studied. The properties *of these compounds are very similar* to the corresponding chloro compounds studied by Levin and Hartung (1942, J Org Chem., 7 408) previously There is considerable resemblence between these compounds and the bromo-oxymethylene ketones which have a similar pentad system. The *study of carbanion formation* in halogen ketones *through nitrosation* has been elaborated

The free isonitroso and nitroso ketones as well as their chloro or bromo *derivatives behave as mild acids. These compounds* have been condensed with an optically active alkaloid (brucine) and the rotatory dispersion of the salts obtained has been studied They all exhibit simple rotatory dispersion in the range—4358 to 6708 A.U and in each case the rotatory power can be expressed by Drude's one term equation namely $\alpha = \frac{K}{\lambda^2 - \lambda o}$ where α is rotatory power for wave length λ and K and λo are constants. Comparison of rotatory power in chloroform and pyridine using similar concentrations and similar temperature *shows that, in general, polar effect of groups* is traceable on rotatory power It has been noticed that the replacement of the electropositive hydrogen by an electronegative group such as -Cl, Br -CH_3 or -CH_2 O causes, in general a decrease in the magnitude of rotatory power Further these effects are more marked in a *solvent like chloroform* which has a low dielectric constant than in pyridine which has a much higher dielectric constant.

STUDIES ON THE EFFECT OF HALOGENS HYDROXY AND BENZAMIDO GROUPS, INDIVIDUALLY AS WELL AS COLLECTIVELY ON THE CONDENSATION REACTIONS OCCURRING BETWEEN HIPPURIC ACID AND HYDANTOIN WITH ALDEHYDES

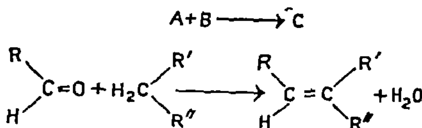
HARBANS SINGH, M Sc.
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The reaction commonly known as Perkin's[11] reaction after the name of its discoverer originated in 1868, when Perkin prepared coumarin by heating together salicylaldehyde sodium acetate and acetic anhydride. It essentially consists in the interaction between two compounds, one containing a carbonyl group and the other a reactive methylene group, an α β unsaturated acid resulting as condensation product. Stuart[12] (1883) introduced the use of malonic acid as a reactant containing a reactive methylene group and by using glacial acetic acid as a catalyst succeeded in achieving the same condensation The next important change was due to Knoevenagel[8 to 11] (1898) who, in place of acetic acid, introduced the use of bases as catalytic agents, such as ammonium salts, aniline or more particularly piperidine The tertiary base pyridine as a catalyst, was first introduced by Verley[14] (1899) and subsequently by others, all using it in full molecular proportion. On the suggestion of Robinson, Perkin Junior (1924) used pyridine (several molecules) with a few drops of piperidine, and this was further followed up by Robinson[15] and Shinoda (1925) who thereby established a classical method for synthesising α β unsaturated acids. Pandya and co-workers did away with the larger proportions of pyridine and showed that a trace of pyridine (0 15 mol.) was not only enough for the success of the reaction but that the yields of the condensation products were better and many a times quantitative and their purity being of a higher standard They also showed (1935 and thereafter) that many other bases, generally tertiary ones, were also efficient catalysts. Workers in this laboratory have used phenyl acetic and p-nitro phenyl acetic acids, as compounds containing a re-active methylene group in condensation reactions. They have not been able to support the claim of Pandya regarding the efficacy of pyridine, and have found the secondary base piperidine more efficient which is also in line with the theoretical considerations (given in the text)

Lock and Baeyer[13] Pandya and co-workers, and R. N Singh and co-workers (in this laboratory) from a study of a very large number of condensations, have shown that the yield and purity of the condensation product is

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governed not only by the catalytic activity of the base used, but by a number of other factors such as the total time of heating the temperature at which the reaction takes place the concentration of the reactants and their chemical nature. Each of these factors have been found to have a far reaching effect on the whole reaction as judged by its velocity and the quality of the yield. In general if the reaction be represented as a reaction between A (aldehyde) and B (a substance with a reactive methylene group)



The nature and quantity of the condensation product C depends not only on the nature of the catalyst and the experimental condition stated above but also on the nature of R, R and R In other words, the reactivity of the oxygen of A and of the two hydrogen atoms of B is not absolute, but is conditioned by the nature and quantity of the base used the varying condition of the experiment and above all by the nature of R, R and R In the case of B if R and R are both—COOH (as in malonic acid) the general reaction goes on very well and the yields are good If however R is still a carboxylic group and R a different group like—CONHPh —CONH $C_6H_4(CH_3)$ or $-C_6H_5$ the position is altered somewhat resulting in the reduced reactivity of the methylene group (as has been shown by Pandya and Ittyerah and R.N Singh and other workers in this laboratory) This has further been borne out by the results obtained in the work presented herewith wherein hippuric acid and hydantoin have been used as reactants containing the reactive methylene group.

When both R and R are like—COOEt, $CONH_2$ or CONHPh, the reactivity of the methylene group falls considerably and the reactions proceed with extreme slowness.

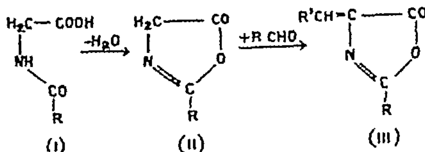
In the case of A it is not only the chemical nature of R but also the exact position of the aldehyde group in the molecule and the imperative necessity of hydrogen are important factors The presence of different groups like Cl, Br I OH NO etc in the benzene ring has again an important effect on the reaction Groups like Cl Br I methoxy nitro and the like have been found to accelerate the reaction resulting in higher yield, while others like hydroxy two nitro groups etc retarded the reaction. These effects have been studied by Lock and Baeyer by using Perkin's method by Pandya and co-workers by using pyridine trace method in the case of malonic acid, and by R N Singh and co-workers in this laboratory in condensation of aldehydes with phenyl acetic and nitro phenyl acetic acids. This study has

further been extended in the present thesis where aldehydes have been condensed with hippuric acid and hydantoin.

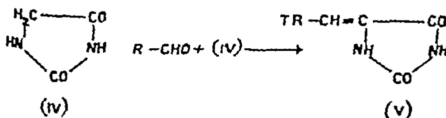
The present thesis deals with the reactions of benzaldehyde, the three monohydroxy benzaldehydes and their haloid derivatives with hippuric acid and hydantoin. The mechanism of the reaction in the case of acylglycine (like hippuric acid) has although been suggested by Carter[6] yet, in view of the experimental results, it needs a fuller theoretical consideration and an experimental support. This is what Carter writes:

"In the azlactone synthesis (from an aldehyde and an acylglycine) uniformly high yields are obtained from aldehydes which give poor results or fail to react in the Perkin condensations. Furthermore the yields from substituted aldehydes do not vary as they do in the Perkin reaction which suggests that the condensation is not the limiting step in the azlactone synthesis. All these data indicate that the intermediate contains an extremely active methylene group and therefore is the azlactone rather than the acylglycine."

In brief Carter[6] suggests that the acylglycine (I) first forms its azlactone (II) as an intermediate. The latter then reacts with aldehyde to give the unsaturated azlactone (III).



The case about hydantoin [7] (IV) has not been commented upon except the mention that its condensation products (V) are obtained in uniformly high yields ranging between 70 and 85 per cent. Nearly of the same order are the yields of the unsaturated azlactones (III) which are formed from commonly substituted aldehydes.



This thesis therefore aims at —

1 a closer theoretical consideration of the mechanism of hippuric acid and hydantoin condensations with aldehydes with a view to understand why uniformly high yields are obtained in comparison with those obtained in Perkin's reaction

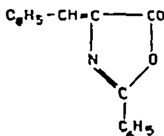
2. providing further experimental evidence by working with aldehydes other than those already worked with a view to see whether in them also the yields remain practically unaffected by the nature of substituents, particularly when the later have been found to exercise definite influence on condensations with other reactive methylene-group substances

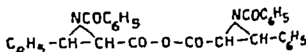
3 the study of the graded hydrolysis of the condensation products.

4 the study of the effect of substituents like hydroxy haloid, benzamido etc on *the condensation reaction its velocity and the yields*

5 the study of the effect of secondary and tertiary bases on condensation reaction

Although Erlenmeyer[4,5] is credited with a vast amount of work on condensation of aldehydes with acylglycines in the presence of sodium acetate and acetic anhydride as in Perkin's reaction it was Plochl[6] who first studied the reaction between benzaldehyde and hippuric acid It is interesting to note that Plochl made these reactants work in the presence of acetic anhydride alone The product obtained by Erlenmeyer[4] was definitely the azlactone (VI) but Plochl describes his product as an anhydride (VII) and shows that its molecular weight agrees with the structure.

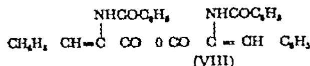




(VI) (VII)

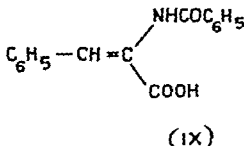
The work of J Plochl with benzaldehyde was repeated and the structure (VII) assigned by him to the condensation product was found to be incorrect. The ethyleneimine structure (VII) is obviously untenable since ethyleneimines are very sensitive to hydrolytic agents (Leighton[11] et el. Jones[7] et el.) Instead the structure of the isomeric compounds, namely the anhydride of a

benzamido cinnamic acid (VIII) has been found to agree with its properties and has thus been assigned.



(VIII)

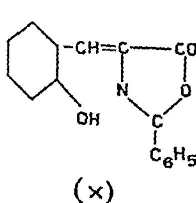
Although Erlenmeyer's azlactone and Plochls anhydride are different substances they both give α -benzamido cinnamic acid IX on hydrolysis.



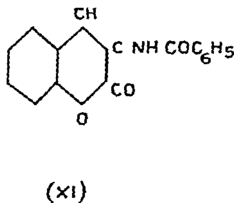
(IX)

Monochloro and 2·4 dichloro benzaldehydes have also been attempted and two types of condensation products are obtained The reactants in the presence of acetic anhydride and sodium acetate gave azlactones while in the presence of acetic anhydride alone anhydride type of compounds are obtained.

P Plochl and Wulfrum[15] studied the condensation of salicylaldehyde with hippuric acid in the presence of acetic anhydride alone at room temperature and obtained an acid anhydride having the aziridine structure But in the presence of sodium acetate and acetic anhydride they obtained a mixture of two compounds which were left unidentified Erlenmeyer (1904) repeated the latter condensation and succeeded in separating the two compounds viz. azlactone (X) and the benzamido coumarin (XI) and reported the melting point of azlectone as 145°C.



(X)

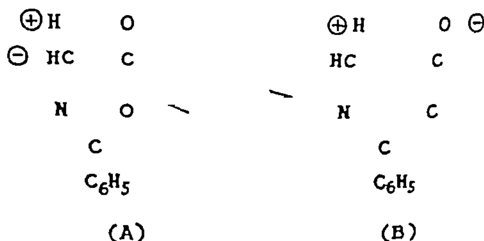


(XI)

During the course of the work submitted herewith the author on repeating these experiments, obtained only the azlactone (X) with m p 145 C after repeated crystallisations of a mixture obtained initially Pure benzamido coumarine could not be separated from the mixture

In the present work the three monohydroxy benzaldehydes and their haloid derivatives have been condensed with hippuric acid as well as with hydantoin in the presence of a mixture of acetic anyhydride and sodium acetate (Erylenmeyer s method[1]) as well as by using acetic anhydride alone in traces and larger amounts Erlenmeyer s method has given a mixture of corresponding azlactones and benzamido coumarins in the case of salicylaldehyde and its halogen derivatives, but only the azlactones could be obtained in a pure form from the mixture while m and p-hydroxy benzaldehydes and their halogen derivatives have given azlactones only When acetic anhydride alone was used in larger proportions as catalyst salicylaldehyde and its derivatives gave acid anhydride type of compounds at room temperature and benzamido coumarins at 100°C, but on using acetic anhydride in traces salicylaldehyde and its halogen derivatives gave benzamido coumarins. P Plochl had assigned aziridine structure to the anhydride which on verification was found to be wrong and a correct structure has now been assigned to the anhydride. The m- and p- hydroxy benzaldehydes and their haloid derivatives in the presence of acetic anhydride in traces as well as larger amounts gave the acid anhydride as the exclusive product.

Carter has suggested that acylglycine before condensation first changes to an intermediate azlactone having an active methylene group. The basic catalyst then helps in the expulsion of a proton from active methylene group leaving the anion which assumes two different electronic forms A and B, and so it will also acquire resonance energy

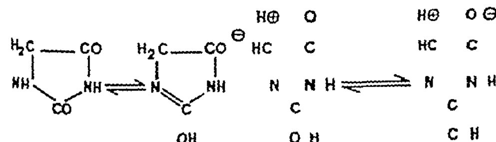


Of these form (A) is conducive to the 'transition state but the form (B) provides a strongly conjugated system. The conjugation starts from the carbonyl carbon and extends upto the carbon carrying the phenyl group. In this case it is further extended to the aromatic ring of the phenyl group although acetylglycine azlactone it terminates here. However in all acylglycine azlactones the strong conjugation of the anion (B) is a great stabilising factor and helps the formation of the anion by expulsion of the proton. This circumstance might account for the great reactivity of the methylene group of hippuric acid azlactone and its condensation with benzaldehyde even without the use of sodium acetate.

Such an explanation although it may show that sodium acetate is not indispensable in this reaction, is not altogether satisfactory. For it is not clear how the azlactone ring of hippuric acid which must remain intact in its reaction with benzaldehyde, is subsequently removed when the anhydride of α benzamido cinnamic acid is formed as is claimed by Plochl. The use of acetic anhydride in Plochl's experiment may explain the formation of an anhydride as the resulting product. But the disappearance of the azlactone ring for the formation of an anhydride finds no explanation.

Benzaldehyde, the three hydroxy benzaldehydes and their haloid derivatives when condensed with hydantoin produced substituted benzalhydantoins. The yields on the whole were found to be good.

The reaction with hydantoin is explained on the same basis as hippuric acid azlactone. Enolisation of hydantoin will produce a double bond between nitrogen and carbon (as it exists in hippuric acid azlactone) and expulsion of proton from the enol form will provide a resonating anion, one form of which has a strong conjugation.



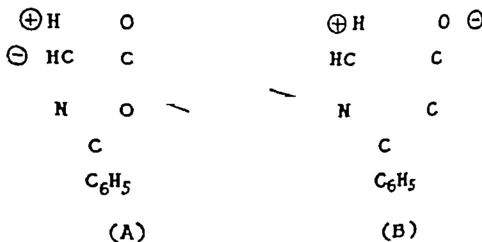
Graded hydrolysis

The graded hydrolysis of the condensation product has given rise to phenylpyruvic acid and its derivatives, which has gone a long way in ejecting the aziridine structure of the condensation product. The use of dilute caustic soda for hydrolysis produced α benzamido cinnamic acids from both azlactones as well as anhydrides. Strong caustic soda (50% solution) or

During the course of the work submitted herewith, the author on repeating these experiments, obtained only the azlactone (X) with m.p 145°C after repeated crystallisations of a mixture obtained initially Pure benzamido coumarine could not be separated from the mixture.

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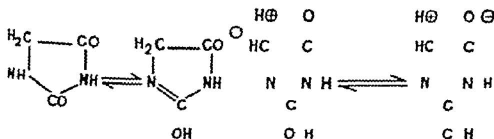


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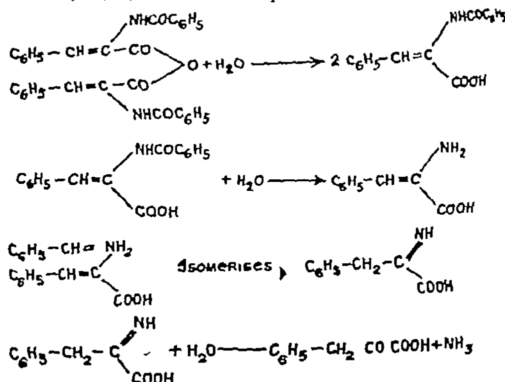
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Graded hydrolysis

The graded hydrolysis of the condensation product has given rise to phenylpyruvic acid and its derivatives, which has gone a long way in rejecting the aziridine structure of the condensation product. The use of dilute caustic soda for hydrolysis produced α-benzamido cinnamic acids from both azlactones as well as anhydrides. Strong caustic soda (50 solution) or

conc. HCl on the other hand acted further with the elimination of ammonia and benzoic acid and the formation of Phenyl pyruvic[1 2 3] acids. The benzamido cinnamic acid *perhaps* gets first converted to α-amino cinnamic acid. The latter contains the *system* $-CH=C(NH_2)-$ capable of tautomerisation to CH_2 $-C(=NH)-$ which should give on hydrolysis $-CH_2-C(=O)-$. According to Borten the entire course of *decomposition* of the anhydride of α benzamido cinnamic acid into phenyl pyruvic acid can be explained as below



The anhydrides from salicylaldehyde when treated with 50% NaOH in the same way gave 3-keto dihydro coumarin

As regards the effect of different groups on the condensation reaction it has been found that when benzamido group replaces a—COOH of malonic acid the *yields are lowered* which is in harmony with the theoretical nature of hippuric acid.

The effect of hydroxy group is generally retarding being maximum in the case of ortho isomer less in the para isomer and least in meta. The halogens have given more or less similar results as has been observed in condensation with malonic or phenyl acetic acids *i.e.* their effect is positive in accelerating the reaction

Both secondary and tertiary amines as catalysts have completely failed in these condensations.

References

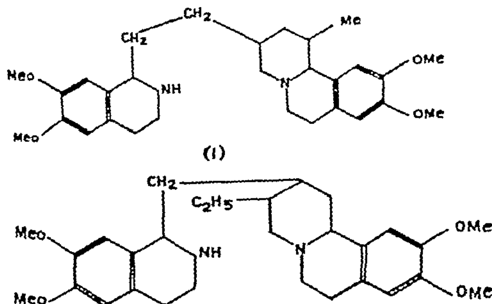
1. Burton. *J. C. S.*, 1935, 1265
2. Burton and Stoves. *J. C. S.*, 1937 402.
3. Carter *Org. Reactions* 3 1946, p. 199
4. Erlenmeyer *Ann.* 1893 175, 1.
5. Erlenmeyer & W. Stadlin. *Ann.* 1904 337 269
6. G. D. Jones. *et. al.* *J. Org. Chem.*, 1944 9 1500. (Annual Reports 1948, p. 217)
7. Johnson. *Org. Reactions*, 1, 1942, p. 211
8. J Plochl. *Ber* 16, 1884 2815
9. Knoevenagel. *Ber* 1898, 31, 2585-2593.
10. Knoevenagel. *Ibid*, 1898, 31 2596.
11. Knoevenagel. *Ibid*, 1893, 31 2768.
12. Leighton. *et. al.* *J. A. C. S.* 1935, 57 2328
13. Lock & Baeyer *Ber* 1939 72, 1064
14. Perkin (Senior) W. H. *J. C. S.*, 1868, 21 53.
15. P Plochl & Wolfrum. *Ber* 18 1183-1188.
16. Robinson & Shinoda. *J. C. S.* 1925 127 1977
17. Stuart, C. M. *J. C. S.*, 1883, 43, 403
18. Verley A. *Bull. Soc. Chim.* 1899 21 143 & 414

SYNTHESIS OF POSSIBLE AMOEBICIDES

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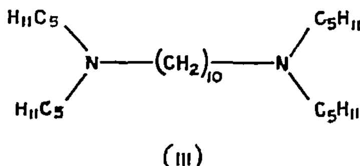
Amoebiasis—a disease caused by a protozoon *Entamoeba histolytica* has been a major health hazard in tropics. The drugs that are available are emetine emetine bismuth iodide chiniofon vioform, diiodoquin carbarsone thiol derivatives of carbarsone oxide milibis and chloroquin. Emetine stands out as the most potent of all the drugs tested clinically and thus remains outstanding in the treatment of amoebic dysentery hepatic abscess and chronic amoebiasis. Nevertheless, it cannot be claimed as an ideal drug because of its cumulative toxic action, its narrow margin of safety and its inability to kill the encysted form of *E. histolytica*. This necessitates the search for a substitute far superior to emetine.

Emetine was formulated by Brindley and Pyman (*J Chem Soc* 1927 1067) as (I) but is now more correctly represented by (II) as shown by the investigations of Robinson (*Nature* 1948, 162, 524) Pailer (*et al.*, *Monatsh*, 1948, 80 94) and Battersby and Openshaw (*Experientia* 1949 5 398 *J Chem.* Soc 1949 3207) Emetine is more active *in vivo* than *in vitro* which furnishes every reason to believe that emetine does undergo some degradational changes in the body though attempts to locate this change have been futile

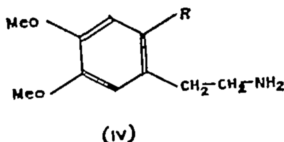


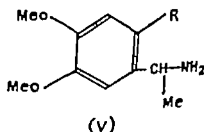
This is an abstract of the thesis submitted and approved for the Ph. D degree of the Agra University for the year 1960

Based on the hypothetical fission of the emetine molecule along different lines of cleavage, several amines have been synthesised and examined for their amoebicidal activity (Child and Pyman, *J Chem. Soc.*, 1929 2010 1931 36 Pyman *Chem. & Ind.*, 1937 56 789 Goodson *et al.*, *Brit J Pharmacol.*, 1948, 3 49 Goodwin *et al.*, *ibid.*, 1948 3, 63 Sugasawa, *J Pharm. Soc Japan*, 1949, 69 8 Hall *et.al.*, *J Chem. Soc.*, 1950 1842 1952, 149 Mahboob and Dhar, *J Sci Industr Res.*, 1955 14B, 1 Osbond *J Chem. Soc* 1951 3464 1952, 4785 1959 2157 Paul and Nitya Anand, *J Sci Industr Res* 1958, 17B, 219 Fancher *et al* *J Amer Chem. Soc.*, 1958 80 1451 Sen and Arora, *Jour Indian Chem. Soc.* 1959 36 349) Only a few compounds especially ι-l-tetra-n-amyldiaminodecane (T A. D D., III) prepared by Pyman (*loc cit*) showed a high order of activity *in vitro* but only feeble activity *in vivo* (Goodwin *et. al.*, *Brit J Pharmacol.*, 1948 3, 44)



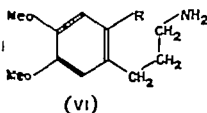
The same line of approach was adopted by Kachru and Pathak (*Jour Indian Chem Soc* 1957 34 611 768) who synthesised β and α-(2-alkyl-4,5-dimethoxyphenyl)-ethylamines (IV and V) as possible hypothetical cleaved fragments of the emetine formula These amines were tested by Kaushiva (*J Sci Industr Res* 1957 16C, 224) who reported the highest *in vitro* activity in hexyl derivatives (comparable to emetine)

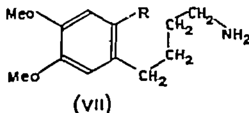




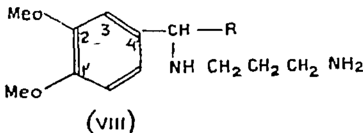
Moreover the β-amines were found to be more active than their α-analogues. Based on these observations the amoebicidal activity would be expected to increase if the amino group is removed still further away from the nucleus by one or two carbon atoms It was considered, therefore worthwhile to synthe-

use compounds having the structures (VI) and (VII) and to examine their amoebicidal activity





Mahboob and Dhar (*J. Sci. Industr. Res.*, *loc. cit.*) have synthesised several diamines containing the two amino groups separated by 3 and 9 carbon atoms. A structure which retains the methoxy moiety of emetine and containing the two basic centres separated by 3 carbon atoms, could be generalized as (VIII) whose amoebicidal activity would be of much interest.

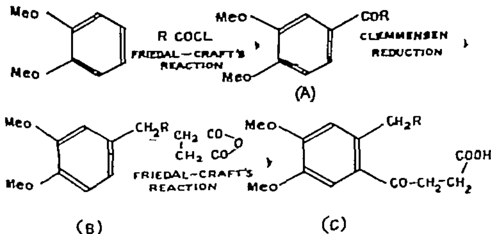


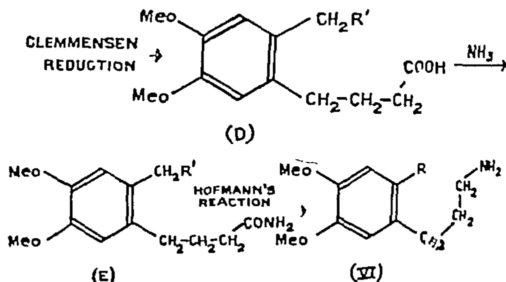
The thesis contains the synthesis of (VI) (VII) and (VIII) described in Parts I II and III respectively

Synthesis of γ—(2-alkyl-4:5-dimethoxyphenyl)-n-propyl-amine (VI) R = CH_3, C_2H_5, n-C_3H_7 and n-C_4H_9.

These amines were synthesised by two routes as shown below —

(a) *SCHEME (I)*

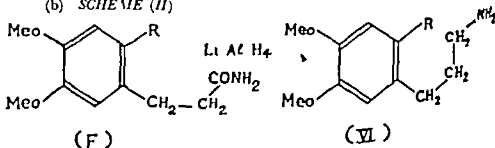




The acylveratroles and their corresponding alkylveratroles were prepared by the procedures adopted by Kachru and Pathak (*loc.cit*) The alkylveratroles were condensed with succinic anhydride in nitrobenzene solution in the presence of anhydrous aluminium chloride to yield the corresponding keto acids in fairly good yields. Solvents other than nitrobenzene *e.g.*, carbon disulphide and *s*-tetrachloroethane, were also tried but the yields were very poor The keto acids were reduced by the Clemmensen reduction in the usual way in toluene solution Subsequent conversion to the amide was achieved by passing a current of dry ammonia through the molten acid at 200° for two hours. The production of the amine (VI) from the amide was done by the application of Hofmann's hypochlorite reaction The amines were examined as their hydrochlorides.

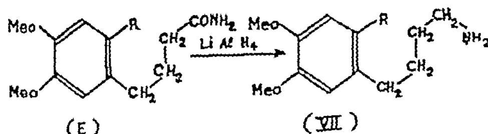
In an attempt to prepare the higher amines of this series with alkyl substitutions as *n*-C_5H_{11} and *n*-C_6H_{13}, it was observed that the reduced acids analogous to γ-(2-alkyl-4 5-dimethoxyphenyl)-*n*-butyric acids, could not be converted to the corresponding amides by either the ammonium salt method or the acid chloride method The hexyl substituted acid was however esterified and then subjected to ammonolysis to yield only 5% of the amide. These reduced acids failed to yield the corresponding amines even by Curtius and Schmidt reactions

(b) *SCHEME (II)*



β-(2-Alkyl-4,5-dimethoxyphenyl)-propionamides (F R = CH_3, C_2H_5, n-C_3H_7 and n-C_4H_9) have been reported earlier by Kachru and Pathak (loc. cit.). These have now been reduced by lithium aluminium hydride in dry ether in good yields.

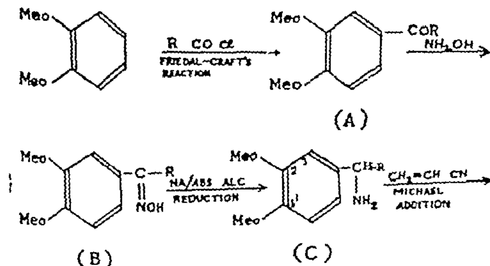
Synthesis of δ-(2-alkyl-4,5-dimethoxyphenyl)-n-butyl-amines (VII) R = CH_3, C_2H_5, n-C_3H_7 and n-C_4H_9.

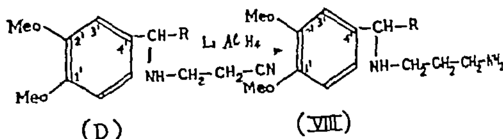


γ-(2-Alkyl-4,5-dimethoxyphenyl)-n-butyramides with alkyl substitutions as CH_3, C_2H_5, n-C_3H_7 and n-C_4H_9 have been obtained as intermediates in the synthesis of γ-(2-alkyl-4,5-dimethoxyphenyl)-n-propylamines. These were converted into δ-(2-alkyl-4,5-dimethoxyphenyl)-n-butylamines by lithium aluminium hydride reduction in anhydrous ether. These were isolated either as picrates or as hydrochlorides.

Synthesis of 1-(4-veratryl) (N-γ-amino-n-propyl)-alkyl-amines (VIII) R = CH_3, C_2H_5, n-C_3H_7, n-C_4H_9, n-C_5H_{11}, n-C_6H_{13}, n-C_7H_{15} and n-C_8H_{17}.

The following route has been adopted for the synthesis of these amines :—





The acylveratroles, prepared by the Friedel-Crafts reaction were converted into the oximes and thence into the corresponding amines (1-(4-veratryl)-alkylamines). These amines were condensed with acrylonitrile by the usual procedure of Michael's addition. The cyanoethyl products could then be conveniently reduced to the desired diamines by lithium aluminium hydride.

Pharmacological findings

Amoebicidal testing (*in vitro*) of some of the selected compounds of the three series was undertaken at the Central Drug Research Institute, Lucknow. The results indicated that δ (2-alkyl-4,5-dimethoxyphenyl)-n-butylamines with n propyl and n butyl substitutions possessed activity comparable to that of emetine. The corresponding γ-(2-alkyl-4,5-dimethoxyphenyl)-n-propylamines were far less active. In the diamines the activity increases with the rise in the molecular weight but the activity exhibited is far from encouraging.

STUDIES IN ION EXCHANGE PHENOMENA AND THEIR APPLICATION TO INDIAN SUGAR INDUSTRY*

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Interest in Ion Exchange and its applications to various fields has increased to an unforeseen extent in the last two decades. Systematic application of ion exchange in sugar industry is also a comparatively recent development. The most important use to which it has been put in this industry is the demineralisation (or deionisation of the clarified juice.)

The primary purpose of demineralisation of sugar solutions is the removal of impurities both mineral and organic derived partly from the soil and partly from the chemical treatment given to raw juice during clarification. The principal inorganic constituents present in the juice are Calcium, Magnesium, Potassium and Sodium sulphates, chlorides, phosphates and lastly silica. The organic constituents are mostly amino acids and such other organic acids as aconitic and itaconic. The inorganic impurities interfere with the process of crystallisation in many ways. The nitrogen compounds are believed to be mainly responsible for the "browning reaction" between glucose and amino acids. The net result of the presence of these impurities is the production of a final molasses whose sugar content is about 35% which cannot be crystallised About 0.2 million tons of sugar is lost in the molasses per year in this country

It is the object of the present work to study the physical and chemical properties of the available ion exchange materials, to make a preliminary selection of the resins that may prove useful for the demineralisation of cane juice and finally to conduct a critical study of the three basic ion exchange techniques viz., direct, reverse and the mixed bed for demineralisation of sugar juices, and arrive at optimum conditions of working of the most suitable technique. The ultimate aim is to establish a method for increasing the sugar production of the country from its present cane resources.

The experimental work was divided into four phases —

(a) Study of resin characteristics

(b) Preliminary investigations on demineralisation in one inch and two inch glass columns.

(c) Bench scale studies in direct and mixed bed techniques in 7 feet × 4 inch transparent perspex columns.

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(*d*) Pilot Plant Trials of demineralisation in 7 ft. × 18 inch transparent perspex columns and subsequent boiling of the juice to obtain crystal sugar

(*a*) The first phase of work included the study of physical and chemical characteristics of a few available ion exchange resins to judge their suitability for treatment of sugar juices. Thirteen resins were selected for this purpose. The strongly acidic resins were Duolite C 25 Amberlite IR 120, Zeokarb 215 and Zeokarb 225 weakly acidic were Zeokarb 226 and Z Amberlite IRC 50 strongly basic were Amberlite IR 410 Deacidite FF and Duolite A 101 weakly basic were Duolite A 7 Amberlite IR 45 and Deacidite E and medium base was Duolite A 30 The physical properties of the resins investigated were moisture content, swelling from dry to wet state, changes in volume in different ionic forms, interstitial volume, apparent density and hydraulic characteristics. This basic physical data was considered necessary for designing of ion exchange plant.

The chemical properties investigated were total exchange capacity acidity and basicity and operating or break through capacity at various regeneration levels. The effect of the variables viz., flow rate, concentration, and nature of the regenerants and the exhaustants on the column capacity was also investigated It was found that whereas the regeneration efficiencies of the strongly acidic and basic resins are in the neighbourhood of 60%, the same in the case of weakly acidic and basic resins approach 100% During regeneration at different concentrations it was found that there is a critical concentration of the regenerant which gives highest regeneration efficiency The optimum doses of respective regenerants which gave high regeneration efficiency and a reasonably high operating capacity were 1 8 meq /ml. and 2.5 meq /ml. of acid for strongly acidic and weakly acidic resins respectively and 1.8 meq/ml. of alkali for both strongly and weakly basic resins. For weakly basic resins, such weak alkalis as sodium carbonate or ammonia solution may be profitably utilized for regeneration. The corresponding capacities at optimum conditions of flow rate and concentration were about 1.2, 2.0 0 9 and 1.2 meq /ml. of strongly acidic, weakly acidic, strongly basic and weakly basic resins respectively The strongly acidic resin Zeokarb 215 possessed very low capacity and was, therefore, rejected.

(*b*) The second phase consisted of preliminary laboratory investigations of the demineralising characteristics of the resins in reverse and direct techniques. In the reverse demineralisation, Amberlite IR 410 and Deacidite FF were used in combination with Amberlite IRC 50 and Zeokarb 226 respectively in the second bed and a mixture of Deacidite FF and Duolite A 7 in the scavenger bed In the direct cycle Duolite C 25 and Duolite A 7 were used Comparison of performances such as ash colour and nitrogen removal and purity rise increase in invert sugar as well as technological and economic considerations show that the direct cycle is preferable to reverse demineralisation. Experiments on determination of colour removing capacities of the available ion exchange mate-

rials viz., Duolite S 30 Duolite ES 102 and Duolite A 7 showed that Duolite A 7 in direct cycle combines a high acid removing capacity with high colour removing ability

(c) The third phase consisted in conducting experiments on demineralisation on a bench scale in 4 inch perspex columns in the direct and the mixed bed technique. In the first thirty experiments Duolite C 25, Zeokarb 225 and Amberlite IR 120 were used along with Deacidite E, Duolite A 7 and Amberlite IR 45 in various combinations in the direct cycle. Flow rates varying between 0.3-0.5 gals/cu. ft./mt. were found to be suitable. Inversion was found to be negligible below 18°C but increased above 20°C. The coefficient of correlation between temperature and increase in invert sugar was found to be 0.68. Regeneration of cation bed with sulphuric acid presented the difficulty of calcium sulphate precipitation in the cation bed. This could be removed either by passing brine or dilute cycle acid prior to acid regeneration. By proper recycling, the consumption of acid could be brought to a minimum of 1 part of acid per part of ash removed. The colour removal due to Duolite A 7 Amberlite IR 45 and Deacidite E was found to be 69 0 % 30 6% and 6.8 % respectively Duolite A 7 was, therefore, selected as the best anion exchange resin. Because of too many variables in this set of experiments, no decision could be taken about the selection of the best cation exchange resin.

The second part of bench scale studies aimed at the selection of the best cation exchanger from among the three resins under as identical conditions of operation as possible. 32 experiments were carried out in this set using Duolite A 7 as the anion exchanger in each case. The purity rise obtained with Duolite C 25, Zeokarb 225 and Amberlite IR 120 was 9 60 7.23 and 7.03 respectively ash removal was 92.7% 91.8% and 85.9 %, nitrogen removal was 90 6 % 87.25% 78.9% organic non-sugar removal was 83 9% 52.5% and 36.5% increase in invert sugar was 2 75% 1.83% and 5.27% These results show that Duolite C 25 is the best cation exchange resin from among the three resins investigated. In separate experiments carried out during off season in 2 columns, on the progress of demineralisation, it was found that the double pass system gives a better quality juice in larger quantity than a single pass system.

The third part of the bench scale studies aimed at evaluation of the mixed bed technique for the demineralisation of cane juice. 28 experiments were carried out in this set using a mixture of Doulite C25 (+20 mesh) and Duolite A 7(−20+40 mesh) in the mixed bed unit and Duolite A 7 (unclassified) in the scavenger bed. The purity rise in this case was 10-13 units, the highest recorded so far with a juice of about 80 purity The ash removal was 95-97% in most cases. The greatest advantage of mixed bed lies in negligible rise in invert sugars Since mixed bed technique was found to be most suitable, other operating data was also worked out. The quantity of juice treated was about 48 gals./cu.ft. of the cation resin. The water requirement was found to be about 160 gals/cu.ft. of the cation bed, and average time required for one complete cycle was

3½ hours The mixed bed technique was, thus, found to be most suitable for demineralisation of cane juice. Experiments on sucrose balance before and after demineralisation of the clarified juice were not conclusive Separate experiments with diluted molasses showed evidence of release of some sugar held up in complexes It was also found during the off season that the once used acid can be, after proper fractionation reused as a measure of regenerant economy

(*d*) The fourth phase of work included experimentation on a comparatively larger scale. The main aim was to demineralise sufficient quantity of clarified juice to be able to boil the same to the stage of final molasses and determine the actual weight of sugar produced. Hot juice from the Experimental Sugar Factory was measured, cooled in a heat exchanger and finally in a refrigeration unit to about 16° 18°C and demineralised in a bigger demineralisation plant comprising of 2 mixed bed columns and two scavenger beds of 18 ins. diameter and 7 feet height. The demineralised juice was concentrated and boiled in evaporators and pans specially designed to handle comparatively small quantity of materials. The massecuite was dropped into laboratory crystallisers, centrifuged and finally sugar crystals were obtained. The mother liquor reboiled with other syrups according to a predetermined schedule Two strikes of white sugar were obtained. The third and fourth strike sugar was brown and was remelted. Four such runs each lasting about a week, of which one was in Direct technique, were carried out in all The purity rise and ash removal were comparable to the figures obtained in Bench Scale Studies. The pilot plant worked for the whole duration of 39 working days without the necessity of cleaning evaporator scales.

A detailed sugar accounting of the materials in process and its comparison with data of the Experimental Sugar Factory for the same period showed that an excess recovery of 10% over the conventional process was obtained in the Pilot Plant The first and second strike sugars were superior to 29 and 28 grade sugars respectively of the Indian Sugar Standards. The quantity of molasses was about one-third of what is obtained in the conventional process. It was low in ash and non-sugar content and was edible. This edible syrup can command higher premium.

Detailed economic estimates for a 2000 ton sugar factory showed that on an additional capital investment of Rs. 25 65 000 a net return of 9 4 is obtained after allowing for insurance, premium, depreciation and interest etc Because of its edible character the molasses of ion exchange process can fetch higher price and can replace white sugar in many food industries. The recovery of aconitic acid and fertiliser values if ammonia is used as the anion regenerant makes the economics much more attractive

The thesis also includes (*a*) Summary of thesis, (*b*) Appendix 1 to relating to cost break up of economic estimates, (*c*) Appendix 6 giving an outline of cane sugar manufacture and (*d*) a typed reprint of a published paper

PHYSICO-CHEMICAL STUDIES ON MUREXIDE

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The thesis is d vided into two parts and consists, in all, of eleven chapters

The chemistry of metal ion indicators such as murexide, eriochromeblack —T etc. has been the subject of recent interest these are used as indicators in volumetric analysis of metallic ions using organic chelating agents such as ethylenediaminetetraacetic acid (EDTA) as titrant. Schwarzenbach s use of murexide in 1946 as indicator specific for estimation of calcium is a landmark in the domain of analytical chemistry this method is so simple that it has soon been adopted for the routine analysis of Ca^{2+} in pure and applied research and technological laboratories. A necessary condition for the use of murexide as indicator for Ca^{2+} appeared to be the maintenance of alkaline nature of the solution, in which the substance *viz* murexide, however tended to undergo decomposition leading to the slow disappearance of its characteristic colour a limitation to its use as indicator No data exist in the l terature on the exact nature of the decomposition the factors responsible therefor etc.

In part I of the thesis are reported the data on detailed investigations on the kinetics of decomposition of murexide or ammonium purpurate (NH P) in alkaline solutions these studies were made by following the decrease with time, in the characteristic absorption of murexide at $\lambda = 250$ 340 and 545 $m\mu$. At these wavelengths the Beer's law was strictly applicable, enabling one to use absorption measurements for the studies of the kinetics of the reactions. The decomposition of murexide in alkaline solutions was proved to be an irreversible reaction the velocity constant of which was dependent on the concentrations of murexide concentration OH and independent of the velocity constant of the bimolecular reaction was obtained by following the reaction in excess of concentration of OH It corresponded to $2\ 44 \times 10^{-}$ at 25 C ($\pm$ 0.2) The kinetics of the reaction was investigated over a wide range of experimental conditions such as ( ) concentrations (C) of murexide, and alkali (*b*) nature of the cation of the alkali (*c*) ionic strength (*d*) temperature and (*e*) dielectric constant of the med um. While ( ) demonstrated the bimolecular nature of the reaction, the results on (*b*) showed the independence of the velocity constant of the cation of the alkali. The energy of activation and the change in entropy of the reaction computed from the results on (*d*) corresponded respectively to 11,210 calories and —26.90 e. s. u. respectively The large magnitude of *k* and the highly negative value of Δ S indicated the ionic nature of the reacting species. The variation of the

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velocity constant with the ionic strength obeyed a relationship due to Bronsted-Christiansen and Scatchard, which gave the product of the charges of the ionic interacting species. Results pointed out that the trivalent purpurate ion obtained from purpurate ion due to loss of two imido protons in alkaline solutions, is one of the reacting species, in addition to OH^- This deduction was amply supported by the data obtained on (d) and (e) this last gave a value of 2.36 A as the radius of the intermediate complex undergoing change. The following mechanism was put forward, which elucidated almost all the observations recorded on (a—e)

(*i*) Murexide dissociates into ammonium and purpurate ions (Fast)

(*ii*) Purpurate ion in highly alkaline solution loses two imido protons to give purpurate ion with three negative charges (Fast)

(*iii*) The triple negatively charged purpurate ion reacts with hydroxide ion to form an intermediate compound (Rate determining)

(*iv*) The intermediate compound is then transformed into colourless products, alloxantin etc. as pointed out by Liebig and Wohler

The studies on the variation of the velocity constant with dielectric constant of the medium appeared to be not only of the theoretical importance (vide supra) but also of marked analytical utility k was 2.40×10^{-1} and 1.07×10^{-1} in aqueous and alcoholic (25% by weight) solutions. These data suggested the use of alcoholic murexide solutions as the indicator for volumetric estimation of calcium by EDTA in alkaline solutions to avoid the fading with time of the characteristic colour of murexide due to inherent decomposition thereof in alkaline solutions (Current Science)

In part II are presented the detailed investigations on polarographic reduction of murexide In accord with the data obtained by Kuhn and others indicating that murexid underwent electrochemical reduction, well defined polarograms using dropping mercury electrode were obtained with murexide. Two distinct reduction waves were noticed the half wave potentials ($E\frac{1}{2}$) of these corresponded to —0 38 and —0 74 volts vs S. C E. in agreement with the data of Ramaiah and Vishnu who reported only one wave with $E\frac{1}{2}$—0.38 volts vs S C E. These authors could not due to paucity of sensitive current measuring instrument record the second wave. Polarographic characteristic like $E\frac{1}{2}$ diffusion current constants $E_{\frac{3}{4}}-E^{\frac{1}{4}}$ etc. were recorded over a wide range of experimental conditions such as supporting electrolytes, concentration thereof, different drop time etc These data proved that the reduction of murexide at dme was irreversible in nature Conductometric studies and diffusion measurements using M Bain and Dawson cell gave a value of 1 07 × 10 and of 9.2 × 10 Cm² Sec respectively for the diffusion coefficient of murexide The analysis of the two waves of murexide appeared possible by employing the obtained value of D This suggested one electron transfer responsible for the occurrence of each wave Examination of the first wave

from the standpoint of Delahay's equation derived for rate controlled irreversible polarograms gave a value of 4.57×10^{-4} for corresponding constant at E =0.146 volts vs N.H.E. The variation of K with E_d obeyed the familiar equation $k = K_0 \; e^{-\frac{\alpha NFE}{RT}}$ where α is the transfer coefficient. This was obtained to be 0.5275

In the end of the thesis two appendices were included. In appendix I were given the data on a few physico-chemical constants of murexide such as the thermodynamic dissociation constant, ionic mobility etc. on which no data exist in the literature. These constants were computed from conductometric data obtained for evaluating the diffusion coefficient of murexide (Chapter 9) pK was 1.564 indicating the behaviour of ammonium purpurate as a strong electrolyte, in accord with appreciable dissociation of purpuric acid (pk of the acid 1.80) The absolute ionic mobility and the ionic radius, calculated from these data corresponded to 38.35 cm. sec $^{-1}$ and 3.85 A° respectively Appendix II reports the data on the determination of stability or formation constant K_f of murexide metal complexes using spectrophotometric method. Application of Job's principle to the absorption of metalmurexide complexes showed the uni unimolecular nature of the metalmurexide complexes. From simple application of law of mass action, equation was derived for computation of the stability constant of the complexes from the spectrophotometric data. Employing this equation, the stability constants of various metal-murexide complexes were calculated and presented in a tabular form. These factors appeared to be of marked importance in developing analytical procedures using murexide (Current Science)

At the end, the reprints of the papers published by the author were included

CRANIAL OSTEOLOGY OF THE INDIAN CLUPEOID FISHES*

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In view of the paucity of work on the osteology of Clupeoid group, the investigations on cranial osteology of Indian Clupeoid fishes were taken up under the guidance of Dr B. M. Sinha, Professor of Zoology Meerut College, Meerut The study besides providing details on the osteology of the fishes, is intended to make possible remarks on the taxonomy of the group as well. A selection of fishes has been made after going through the different classifications on the group. Eight different fishes, one from each group of family Clupeidae erected by Day (1878) have been chosen While making a selection, stress has been laid on the easy procurability and typical form of the fish. The types taken up for study are *Hilsa ilisha, Thrissocles purava Nematalosa nasus Dussumieria acuta, Albula vulpes Elops saurus Megalops cyprinoides* and *Chanos chanos*

The script runs into fourteen chapters, of which the first three deal with the introduction historical resume and material and methods. Chapters four to eleven incorporate the observations on the eight different skulls and the last three chapters include discussion, bearing on taxonomy and bibliography In the end is a list of publications from the author

The observations on the study undertaken may be summarised as follows

Hilsa ilisha (Ham.)

The skull is edentulous and its large gape is formed by both the premaxillae and maxillae. The cranium bears the anterior fontanelle, temporal fossae, preepiotic fossae and auditory fenestrae. The parietals are small and separated by the supraoccipital The sphenotic and pterotic bones project into spines. The orbitosphenoid is enlarged and meets the parasphenoid and lateral ethmoids to form the bony intero bital septum. The basisphenoid is very small and does not bisect the mouth of the myodome. The parasphenoid forms the mid-ventral keel and its two posterior wings extend beyond the occiput. The prootic and pterotic bones bear bullae for the vesicles of the swim bladder

The maxilla bears a pair of supramaxillaries. The axis of the hyomandibula is nearly vertical and its two heads articulate with the sphenotic and pterotic. The sesamoid angular is present and retroarticular is absent. There are only six branchiostegal rays. The first pair of pharyngobranchials suspend

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the branchial skeleton from the parasphenoid. The first arch bears a suprapharyngobranchial and the fourth epibranchial forms the pharyngeal pocket.

Thrissocles purava (Ham.)

The skull has a large gape formed by both the premaxillae and maxillae. The cranium bears a mid-dorsal ridge and a pair of fontanelle in front of the supraoccipital. The preepiotic fossa is larger than the temporal fossa. The frontal is depressed into a trough, which is covered by a pair of transverse bony bridges. The parietals do not meet in the middle line and the usual prootic and pterotic bullae are absent. The wings of the parasphenoid are reduced to small processes, which fail to reach the occiput. The basisphenoid is small and it does not bisect the mouth of mydome. The small orbitosphenoid fails to articulate with the parasphenoid and lateral ethmoids. The opisthotic is absent.

The maxilla is very long and reaches the pectorals behind. Only one supramaxillary is present and the subopercular is smaller than the interopercular. The axis of the hyomandibula is inclined backwards and its two heads articulate with the sphenotic and pterotic. The symplectic is absent and there are twelve branchiostegal rays. The vomer palatine ectopterygoid, entopterygoid, metapterygoid, dentary basihyal and bones of the branchial skeleton are all toothed.

Nematalosa nasus (Bloch.)

The skull is edentulous and the small gape is contributed by the premaxillae alone. The cranium bears the anterior fontanelle temporal fossae preepiotic fossae and auditory fenestrae. The parietals are separate and the sphenotic and pterotic bones bear spines. The prootic and pterotic bear the usual bullae and the small basisphenoid fails to bisect the mouth of myodome. The orbitosphenoid meets the lateral ethmoids only. The parasphenoid forms a keel and its two wings extend beyond the occiput.

The axis of the hyomandibula is inclined forwards its anterior head articulates with the prootic and sphenotic, while the posterior head fits into the depression on th prootic sphenotic and pterotic. The maxilla is small and bears only one supramaxillary. A small retroarticular is present, but the sesamoid angular is absent. The subopercular is smaller than the interopercular. There are only fi branchiostegal rays and the fourth epibranchial forms the pharyngeal pocket. A suprapharyngobranchial is present.

Dussumieria acuta Cuv. & Val.

The skull has a small gape contributed by the premaxillae and maxillae. The preepiotic fossae are better developed than the temporal fossae and the auditory fenestra are present. The usual prootic and pterotic bullae are found. The left frontal slightly overlaps the right and the parietals are separate. The

orbitosphenoid meets the lateral ethmoids only and the small basisphenoid does not bisect the mouth of myodome. The parasphenoid is not keeled but its two laminate wings extend beyond the occiput.

A pair of supramaxillaries and a small retroarticular are present. The axis of the hyomandibula is inclined forwards and its two heads articulate with the sphenotic and pterotic. The vomer palat ne ectopterygoid, entoptery goid, premaxilla, maxilla, dentary basihyal and bones of the branchial skeleton are toothed. There are fourteen branchiostegal rays.

Albula vulpes (Linnaeus)

The skull has a small gape formed by the premaxillae alone. The cra nium is devoid of the anterior fontanelle temporal fossae, preepiotic fossae and auditory fenestrae. The parietals meet in the middle line The subtem poral fossae and posterior temporal fossae are present. The ethmoid projects ahead of vomer and the left frontal slightly overlaps the right. The basisph noid is well developed and bisects the mouth of myodome. The orbitosphen id supports the interorbital septum partly but fails to reach the parasphenoid and lateral ethmoids. The processes of the parasphenoid are small and do not extend beyond the occiput The usual bullae on the prootic and pterotic are absent.

The two premaxillae do not meet each other The axis of the hyoman dibula is directed forwards and its single head articulates into the depression formed by the sphenotic and pterotic. The interopercular is smaller than subopercular The symplectic gives off an extension which supports the meta pterygoid The vomer parasphen id, palatine, ectopterygoid entopterygoid premaxilla, dentary basihyal and bones of the branchial skeleton are toothed. There are twelve branchiostegal rays. The branchial keleton is attached to the parasphenoid through ligaments and not by the first pair of pharyngobranchials.

Elops saurus Linnaeus

The skull has a small gape formed by th premaxillae and maxillae. The cranium is devoid of temporal fossae, preepiotic fossae and anterior fontanelle. The subtemporal fossae are present and the posterior temporal fossae form deep chambers over the brain case, which do not meet each other The auditory fenestra is closed by thin membrane and is formed by the parasphenoid as well. The usual bullae in th prootic and pterotic ar bsent The parietals meet in the middle line and the left f ontal overlaps the right. The basisphenoid is large and bisects th mouth of myodome. Th parasphenoid is not keeled and its two small processes articulat with the basioccipital and terminate on reaching the occiput.

The premaxillae join into a symphysis in front of the ethmoid. Each maxilla bears a pair of supramaxillaries. A sesamoid angular is present and retroarticular is absent. The vomer parasphenoid palatine ectopterygoid, entopterygoid premaxilla, maxilla, dentary basihyal and bones of the branchial skeleton are toothed. The axis of the hyomandibula is directed forwards and its two heads articulate with the sphenotic and pterotic. There are twenty nine branchiostegal rays and a median intergular plate The first pair of pharyngobranchials is suspensory and the second pair gives attachment to the spicular bones for the attachment of the branchial skeleton with the cranium.

Megalops cyprinoides (Brouss.)

The skull has an oblique cleft of mouth with the gape formed by the premaxillae and maxillae. The cranium is devoid of anterior fontanelle temporal fossae and preepiotic fossae The posterior temporal fossae are very deep and meet over the brain case forming large chamber The auditory fenestrae lie very near the occiput and are closed by thin membranes. A pair of deep subtemporal fossae are also present. The processes from the ethmoid and lateral ethmoid form a characteristic nasal pit The vomer projects a little ahead of ethmoid and the left frontal overlaps the right The parietals join in the middle line and the reduced orbitosphenoid does not join the parasphenoid and lateral ethmoids. The basisphenoid is well developed and bisects the mouth of myodome The usual prootic and pterotic bullae are absent. The parasphenoid is not keeled and its two small processes do not extend beyond the occiput.

A pair of supramaxillaries is present, while the sesamoid angular and retroarticular are absent The axis of the hyomandibula is inclined forwards and bears the usual two heads. The vomer parasphenoid, palatine ectopterygoid ent pterygoid, premaxilla maxilla, dentary basihyal and bones of branchial skeleton are toothed. There are twenty four branchiostegal rays and a well developed intergular plate The second pair of pharyngobranchials bear spicular bones.

Chanos chanos (Forsk.)

The sk ll is edentulous and its small gape is formed by the premaxillae alone The anterior fontanelle temporal fossa preepiotic fossae and auditory fenestrae are absent Th vomer projects in advance of the ethmoid. The pa tals a separate but their thin processes meet over the supra occipital The orbitosphen d and bas sphenoid are absent and th pr jec tions of parasphenoi f l to reach the occiput. The prootic and pterotic bullae are absent. Th supraoccip tal spine splits into a number of bony filaments The exoccipitals project behind into processes, which extend over a few anterior vertebrae

The supramaxillaries and sesamoid angular are absent, but the retroarticular is present. The metapterygoid fails to meet the quadrate and the dentary abruptly becomes thin in front. The quadrate gives off a long spine to support the symplectic and the hind part of ectopterygoid applies on the inner face of the quadrate. The axis of the hyomandibula is inclined forwards and its single head articulates with the depression on the sphenotic and pterotic. The symplectic is long and supports the metapterygoid. There are only four branchiostegal rays. The front end of each of the first three pairs of epibranchials gives off a backwardly directed process for the support of the pharyngobranchial of the subsequent arch. The epibranchials of fourth arch give off elongated limbs to attach the branchial skeleton with the cranium.

Discussion

The discussion has been done in light of the previous work on the osteology of fishes. A review of the literature revealed the paucity of work on the osteology of this group. Except of the outstanding work of Ridewood (1904) on the osteology of Clupeoid fishes, Chapman (1941-48) on a number of Isospondylous fishes, Phillips (1942) on *Sardinops caerulea*, Joshi & Bal (1953) on *Coilia dussumieri* and the author (1959-60) on *Setipinna phasa*, *Hilsa ilisha* and *Coilia dussumieri*, nothing of importance is on record.

Bearing on Taxonomy

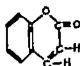
On comparing the eight skulls among them, a number of features of resemblance and difference have been noted. The resemblances between the skulls suggest the placement of *Hilsa* and *Nematalosa* in one family Clupeidae and *Thrissocles* and *Dussumieria* in another family Engraulidae. *Albula*, *Elops* and *Megalops* are found to be distinct from the remaining fishes and are considered more primitive. Of these fishes, *Elops* and *Megalops* which resembl greatly may be placed in one family Elopidae and *Albula* in another family Albulidae nearer to Elopidae. *Chanos* bears certain resemblances to the members of the family Clupeidae, but has a number of features exclusive to itself. It has, therefore, been considered appropriate to place it in a separate family Chanidae nearer to family Clupeidae. *Thrissocles* and *Dussumieria* have many more points in common with *Hilsa* and *Nematalosa* than with *Chanos*. The family Engraulidae may therefore, be placed nearer to family Clupeidae than family Chanidae.

The present reflections are mere suggestions borne out of the study, rather than confirmed directions. The suggestions have been made with a view to be helpful to those who get interested in the taxonomy of this fascinating group and include more fishes in their field of study.

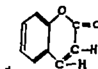
STRUCTURE OF COUMARIN MOLECULE

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Several constitutional formulae have been assigned to coumarin molecule however the one assigned by Strecker Fittig and Tiemann (A) has been universally accepted (vide Hugo Schiff[1]) as it is in complete accord with the known chemical reactions. According to this, coumarin is regarded either as a lactone derived from o-hydroxy cinnamic acid or a heterocycl c compound formed by the fusion of benzene and 1 2 pyrone ring



(A)



(B)

On the basis of fixation of double bonds[2-4] Seshadri and Rangaswami[5,6] have concluded that although the normal structure of coumarin is (A) yet the occurrence of the structure (B) must be taken into account, since some reactions show that the nuclear bonds are not completely fixed. Thakore and Shah[7] consider that according to the resonance theory[8] the actual structure of coumarin can be neither (A) nor (B) but some structure intermediate between the two. Since the structure with a double bond common to both rings possesses lower energy due to less distortion of valence bond the actual structure resembles (A) more than (B)

Murti and Seshadri studied the Raman spectra of coumarin (1) in the solid state and (2) in solutions in different solvents Of the three C=C frequencies, namely 1570 cm^{-1} 1610 cm^{-1} and 1625 cm^{-1} observed by them the two lower ones have been assigned to the C=C linkages in the benzene ring and the third one to the ethylenic double bond present in the pyrone ring, since the Raman shifts observed for benzene are 1586 cm^{-1} and 1606 cm^{-1} and that for ethylene is 1620 cm^{-1} These workers observe that though the pyrone double bond is situated in a ring it is highly reactive and hence its resemblance to ethylene deduced from Raman frequency is justifiable. This view does not appear to be wholly true since the addition reactions[14,15] do not take place readily Even on bromination the addition product formed is highly unstable and readily loses a molecule of HBr yeilding a mono bromo derivative.

The three Raman frequencies (1570 cm^{-1} 1610 cm^{-1} and 1625 cm^{-1}) observed by Murti and Seshadri can be compared with those observed (1577 cm^{-1} 1596 cm^{-1} and 1630 cm^{-1}) by Benel Kastler and Rousset [4] for naphthalene crystals. One would therefore be led to consider that the resonance in cou-

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marin may be similar to that in naphthalene. This view receives support by subsequent work of Volkenshtein and Syrkin[15] who observed that the Raman spectrum for coumarin resembles that of naphthalene and coumarone.

The problem of structure of coumarin can also be attempted by the study of the diamagnetic property of the coumarin molecule. In this connection the calculations given in the following paragraphs have been made. The molecular diamagnetic susceptibility (χ_m) of coumarin purified by repeated crystallisation and measured by Gouy's method, has been found by the author[16] to be 83.0 ± 0.3. This value compares favourably with the value 82·5 found previously by Pacault[17]. All values of susceptibilities given in this paper have been expressed in terms of 1×10^{-6} c.g.s. units.

1 Constitutive correction factor for α benzopyrone ring

Using Pascal's values[18] for the atomic susceptibilities of carbon, hydrogen and oxygen, the value of the constitutive correction factor (λ) for α benzopyrone ring has been deduced from the observed value of χ_m for coumarin, assuming Pascal's additivity law and found to be 3·47 indicating an exhaltation in the susceptibilities of the constituent atoms. Since such exhaltations have usually been found for aromatic compounds the above calculation indicates the aromatic nature of coumarin molecule.

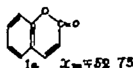
2 Pink and Ubbelhode criterion

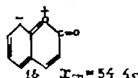
Since aromatic character implies the presence of a pool of electrons with orbital radii extending over the whole of the aromatic region, Coulson[19] and Ubbelhode[20] argue that the presence of giant or aromatic orbit should give rise to substantially higher diamagnetism per electron than shown in the case of orbits bound to single atoms. Neglecting the complication in applying this criterion of aromatic character arising from the possibility of superposed paramagnetism independent of temperature, Pink and Ubbelhode[21] have been able to distinguish between aromatic rings of carbon atoms and cyclic system of conjugated double and single bonds, from the values (87.35 for the former and 57·65 for the latter) calculated for them, using Pascal's constants. Since the value (83·0) for χ_m observed for coumarin approximates to that calculated for aromatic system, the criterion employed by Pink and Ubbelhode indicates that the coumarin ring is aromatic in character.

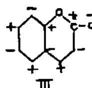
3 Application of Gray and Cruickshank's method[22]

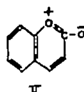
Assuming that the coumarin molecule consists of a benzene and a pyrone ring and that there is no electronic interaction between the two rings, the molar susceptibility for this structure has been calculated by using Pascal's constants for the benzene ring and the susceptibility of the pyrone ring calculated by the Gray and Cruickshank's method and is found to be 71·71. This value is much lower than the observed value (83·0) indicating that the pyrone ring cannot be treated as a separate entity.

Assuming an interaction between the π-electrons of the benzene and the pyrone rings the following resonance structures can be written for the coumarin molecule









On polarisation of double bonds structure Ia, Ib and II give rise to internal ionic structure III. Assuming equal contribution between (i) structures III and Ia Ib and II together (ii) structures II and Ia and Ib together and (iii) structures Ia and Ib, the contributions of structures Ia, Ib, II and III to the susceptibility of coumarin would be 12½% 12½% 25% and 50% respectively The susceptibility for coumarin calculated on this basis is found to be 82 74 which is in good agreement with the observed value 85 0 hence this method confirms the aromaticity of the coumarin molecule. Such confirmations may help to remove the doubts, since in case of aromatic molecules 50% contribution has to be taken into account due to internal ionic structure which is highly unstable, sometimes expressed on Gray and Cruickshank s method which though theoretically not quite sound, does help in establishing the character of a molecule

4 *Conclusions*

Thus the study of the Raman effect and the magnetic property establishes independently the aromatic character of coumarin molecule The aromatic behaviour of coumarin points out that there is an interaction between the π-electrons of pyrone and benzene rings and the double bonds between various carbon atoms are only partially and not rigidly fixed.

5 *Behaviour of C=O group*

Murti and Seshadri[9] have also measured the Raman frequencies for C=O group in coumarin When dissolved in carbon tetrachloride the Raman line is observed at 1742 cm^{-1} which has been ascribed to the normal C=O bond In solution in chloroform and methyl alcoh l the line is observed at 1720 cm^{-1} which has been ascribed to hydrogen bonding between the solute and the solvent In solid state two lines corresponding to 1708 cm^{-} and 1731 cm^{-1} have been observed and assigned to intermolecular hydrogen bonding

The existence of hydrogen bonded structure in coumarin in the solid state seems untenable for the following reasons

(1) On the consideration of electronegativities[23] the ionic character of C-H bond is small and hence insufficient to permit it to attract an adjacent negative atom (in this case oxygen) with appreciable force.

(2) The molecular weight of coumarin determined by the author by (i) freezing point method, using benzene as the solvent, and (ii) Rast's method, is found to be 144.7 and 149.9 respectively. These correspond to the molecular weight (146) computed for the single molecule of coumarin.

(3) Rau[11] and lately Jatkar and Deshpande[25] have shown that the dipole moment of coumarin is independent of temperature and concentration this would not be so in case H-bond is present.

(4) According to Pauling[26] the dielectric constants of H-bonded substances are higher than those of substances which have no such bond. He has also drawn a graph of dielectric constants against dipole moments of substances having H bonded structures. The value of dielectic constant read from the graph for the dipole moment (4.5D) for coumarin[11, 27, 28] should be very high (>> 100). The actual value observed by Kulkarni[29] is 34 at 70°C, which is much lower than the value expected for H-bonded coumarin.

It is interesting to observe from the resonating structures discussed earlier that the behaviour of the C=O group is very much similar to that observed in the case of carboxyl group namely :



This shows that C=0 group in coumarin ring is not ketonic in nature. This conclusion is supported by the fact that no addition reactions, specific for ketone, take place with coumarin. The splitting of Raman frequency for C=0 group for coumarin in the solid state observed by Murti and Seshadri may be assigned to the presence of nucleonic C-0 present in the resonating structures Ib and II. This indicates that the bond order of the CO group would be nearer one rather than two that is the behaviour of the bond by which oxygen atom is attached is essentially similar to that of a single bond. The recent work of Jatkar and Deshpande indicates that the observed value for the dipole moment for coumarin is in good agreement with the value calcuated for the structure II.

Summary and Conclusion

The coumarin molecule is aromatic in nature. The C=0 group is not essentially ketonic its behaviour is similar to the CO group present in the carboxyl group. The conclusion regarding the existence of H bonding in coumarin molecule in solid state is untenable.

ACKNOWLEDGMENTS

The author wishes to express his indebtedness to Dr Mata Prasad and Prof S. M. Mehta for their help and guidance. He also wishes to thank Dr G R. Kanekar for helpful suggestions and discussions, and the Government of Bombay for the award of a Research Fellowship for the first two years of the period of his study

REFERENCES

1 Schiff — Ber., 1872, 5, 665
2. Mills and Nixon — J Chem. Soc., 1930, 2510
3. Fieser and Lothrop — *J Amer Chem. Soc., 1935 57 1459*
4 Baker and Lothian — J Chem. Soc., 1935 628
5 Seshadri and Rangaswami — Proc. Indian Acad. Sci., 1938, 7A, 8
6. Seshadri and Rangaswami — *Ibid.*, 1941 14A, 547
7 Thakore and Shah — J Univ Bombay 1947-48, 16 38
8. Pauling and Sherman — J Chem. Phys., 1933 1 605
9. Murti and Seshadri — Proc. Indian Acad. Sci., 1938, 8A, 519
10. Palfray — J Amer Chem. Soc., 1941 63 3540
11. Palfray — Bull. Soc. Chim. 1940 7 401
12. Seshadri and Rao — Proc. Indian Acad. Sci 1936 4A, 163 630
13 Seshadri — J Chem. Soc., 1928 166
14. Benel, Kastler and Rousset — Compt. rend., 1940 211, 595
15. Vol' kenshtein and J Syrkin — J Phys. Chem. (U S. S. R.) 1939 13 918; Acta Physicochim (U S S. R.) 1939 10, 677
16. Mathur — Trans Faraday Soc., 1960, 56, 325
17 Pacault — Ann. Chim., *1946 1 (12) 527*
18. Pascal — Ann. Chim et Phys 1910, 19 5 1912, 25, 289 1913 28, 218
19 Coulson — Quart. Rev 1947 1 144
20. Ubbelohde — Nature 1933 132, 1002
21 Fink and Ubbelohde — Trans. Faraday Soc., 1948, 44, 708
22. Gray and Cruickshank — *Ibid.*, 1935, 31, 1491
23. Pauling — 'Nature of Chemical Bond' Cornell Univ Press ed. 1948, p. 294
24. Ram — Proc. Indian Acad. Soc., 1936, 4A, 695
25 Jatkar and Deshpande — J Indian Chem. Soc., 1960, 37 19
26. Pauling — Nature of Chemical Bond' (Oxford Univ Press ed. 1952) p. 292 and also Kumler J Amer. Chem. Soc., 1935, 57 600
27 Syrkin and Vasiliev — Acta Physicochim (U S. S R) 1937 6, 639
28. LeFevre and LeFevre — J Chem Soc., 1937 1088
29 Kulkarni — A. I. I. Sc. Thesis, Banglore

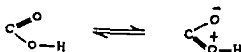
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SUMMARY AND CONCLUSION

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CONTENTS

OPTICAL ACTIVITY AND CHEMICAL CONSTITUTION

Part II—Physical factors influencing optical rotatory power

O N Perti, M Sc. D Phil

Professor of Chemistry Th. D S B Government College, Naini Tal

Physical factors which are found to influence optical rotatory power are

1. Temperature
2. Solvent and concentration
3. Wavelength of light used

Temperature The influence of temperature was noticed by Biot 1844[a,b]) in his investigation of the rotatory power of tartaric acid. Later Gernez (1864[a,b]) studied the influence of temperature on the rotatory power of three essential oils over the range from 0 to 160 for five Fraunhofer lines. He observed a decrease in rotatory power which was almost constant for the five different wavelengths which after taking into account the expansion of the liquids was found to be in the ratio 1 47 1 f oil of orange and oil of bigarade but in the ratio 1.02 1 in the case of oil of turpentine. A much more comprehensive investigation was carried out by Krecke (1872) who examined the rotatory power from 0 to 100 of tartaric acid and a series of metallic tartrates. In case of tartaric acid he used aqueous solutions containing 50 %, 40 % 20 % and 10 % tartaric acid Some of his data is given below

Tartaric acid 50% at	Fraunhofer lines				
	C	D	E	B	F
0°	5 641	6 425	5 771	5 746	5 635
100°	13.253	15.253	17 819	18 467	19 671
Ratio of increase	2.3	2.5	2 7	3 1	3 4

Tartaric acid 40% at	Fraunhofer lines				
	C	D	E	B	F
0°	4.570	5 459	6 702		7 082
100°	15.392	17.506	19 924	20.558	22.689
Ratio increase	3 2	3.2	3 0		3.2

This data indicates that the rotatory power has increased approximately three-fold in the range from 0 to 100° In case of salts of tartaric acid such

*Part I and Part II of this review were prepared under the guidance and in collaboration with late Dr B. K. Singh, Sc. D (Dublin) Sc. D (Cantab.) in project sponsored by U P Scientific Research Committee.

as potassium tartrate ammonium tartrate, sodium tartrate potassium sodium tartrate and tartar emetic he records that the specific rotatory power of potassium salt decreases slightly whilst that of the sodium salt increases slightly when the temperature is raised from 0 to 100° Rochelle salt showed a definite increase of rotatory power with rise of temperature but tartar emetic showed a decrease in rotatory power All this earlier work has been reviewed by Landolt (1879)

Pictet (1882) found that in the case of methyl D-tartrate the specific rotation increased from +2.14 at 20° to +6 00 at 100° Le Bel tried to explain this change by saying that it has a simple molecule at 100° but probably polymerises at lower temperature He tried to find out the role of polymerisation by determining molecular weights and was led to the conclusion that at lower temperatures the molecules undergo a kind of internal congelation Frankland and Mac Gregor (Frankland 1894) however found that in case of ethereal salts of glyceric and diacetyl glyceric acids, the temperature had an influence on rotatory power but it is independent of any molecular polymerisation.

That the influence of temperature may even lead to a reversal of sign of rotation (inversion) was also noticed early by Thomsen (1882) in the case of malic acid at certain concentrations Cook (1897 b) found similar results in cases of xanthogen succin c acid and aspartic acid In the case of aspartic acid at 20° the rotation for sodium light was +4 36° and at 90° it was —1.86° whereas at 75° the rotation observed was zero

Recently M P Balfe M K Hargreaves and J Kenyon (Balfe 1950) have shown that the rotation of a substituted propylene oxide (—) — 1 2-epoxy 3-phen xy propane varies widely with change of temperature Such a variat on can not be explained in terms of rotational configuration on which most f the m dern theories of optical activity are based such as those given I Kuhn (1930) B rn (1930) d Mallemann (1927) and Boys (1931) nor can it be explained b an expression such as the Kirkwood equation (193-)

If one takes into account th observations of various workers it would be apparent that no general tatement an be made relating the change of rotation with temperatu Ea h ompound must be studied separately and the change in rotation w th t mperatur n p t ular sol ent expressed graphically or in terms f quat on wl h would be lim ted in its application to that substance only It may be ment oned h re that the temperature effect in case of solutions f ptic all a t solutes in ina tive solvents is intimat ly c nnected with the phen n ena f diss iat m s ociatio n and s l ation wl ich are discussed later Low ry 193 ha mmed p most f the results If we tak the temperature rotat f r an tive ompound at l igl dilution in non-polar solvents as a sta d l f ompa w r th corresponding d gram for a h g l polar solvent will n general be epresented by a curv lying e ther at below the standa d At low t mperatures h wever the degree f d p le are

ciation between solvent and solute will be greater than at high temperatures consequently the displacement in rotatory power due to the association will also be greater at low temperatures. Under these conditions t can be suggested that, in general, at higher temperature the rotation curves of a substance in polar and non-polar solvents will tend to converge This is illustrated by a number of cases studied by Rule and others. Patterson (1908a,b) noticed the convergence of temperature rotation curves for highly polar and non-polar solvents in the case of ethyl tartrate and Rule got similar results in derivatives of tartaric acid, (—)—menthol and (—)—benzoin (See Lowry 1935)

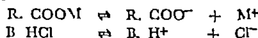
From the foregoing brief account of the influence of temperature on optical rotation it follows that when optical rotatory power of substances is compared, the comparison should preferably be carried out at simila temperatures to avoid any possible complications. There is l ttle doubt that temperature effects the degree of dissociation or association of the active compound When optically active substances are examined as such if they are liquids or in solutions the optical rotatory power would depend to a certain extent upon the degree of dissociation or association These are considered in the next section.

Solvent and Concentration It was Biot (1838) who first noticed the effect of concentration on optical rotatory power of solutions and suggested a linear relationship, $\alpha = A + Be$ where α is rotation and proportion by weight of solvent and A and B are arbitrary constants. Biot (1844a,b) himself later noticed that aqueous solution of tartaric acid shows an increase in specific rotation with a decrease in concentration and that campho in alcohol and acetic acid exhibits the reverse phenomen n. This clearly indicated that the ffect of concentration and solvent on rotatory power is a very important physical facto He also tried to express the influence of concentration by a hyperbolic expression of the type $\alpha = + \frac{Be}{1+Ce}$ where A, B and C were constants.

It may be mentioned that several later workers tried to study the effect of concentration only on optical rotatory po er ignoring the role of solvent. Patterson (1901 1902, 1908a,b 1910) studied the specific rotations of ethyl tartrate at different dilutions in different solvents. Similar studies on a more elaborate scale were carried out by Lowry and Austin (Lowry 1922) in th case of tartaric acid in aqueous solution at 20° fo six d fferent wavelengths. These workers found that th effect of concentration cannot be expressed by a formula (linear or hyperbolic) which had been proposed by Biot earlier Recently M. K. Hargreaves (1953) examined th rotatory power of (—)-octane-2-ol in different concentrations but could not arrive at any definite relationship between concentration and optical rotatory power

There is no doubt that concentration has an important influence on rotatory power of solutions containing optically active compounds. It is intimately

connected with the phenomenon of dissociation association and solvation which are concommitant when an optically active compound is examined in solution The earliest indication of this relationship was obtained by Landolt (1873) and Oudemanns (1876) *They pointed out that solutions of potassium* and ammonium salts of (+)-tartaric acid on the one hand and salts of different acids with quinine on the other hand approached a constant value of molecular rotation with dilution This result is a natural consequence of the theory of electrolytic dissociation to which attention was first drawn by Hädrich (1893). According to him a dilute solution of a salt of an optically active acid (R.COOM) owes its rotation entirely to the optically active anion and in case of the salt of an optically active base (B HCl) to optically active cation In solution we would have



$$R.COOM \rightleftharpoons R.COO^- + M^+$$
$$B\,HCl \rightleftharpoons B.H^+ + Cl^-$$

It is obvious that in concentrated solutions both the undissociated salt and the anion (in the case of active acid) or cation (in the case of active base) would effect the plane of polarized light As the solution becomes more and more dilute there would be gradual disappearance of the effect of undissociated salt and the effect of ions would predominate

Hädrich investigated salts of alkaloids such as morphine quinine cinchonine brucine and strychnine In each case he found that in sufficiently dilute solutions the value of molecular rotation approached a constant. Similar results were also given by boryl, arsenyl and antimonyl tartrates. Confirmation of the views of Hädrich was obtained by the studies of G Carrara and Guenara (1894) and Walden (1894) Similar studies were carried out on metallic salts of Reychler's acid by Thomas and Jones (1906) Graham (1912) who obtained a mean value of $[M]_D = 52^{\circ}$ for (+)-camphor β-sulphonate ion. Singh and Perti (Singh *et al* 1944 1945) noticed that primary amine salts of (+)-camphor β-sulphonic acid are more or less fully ionized in 1 to 4 per cent aqueous solutions at 26 to 35° and give a value of $[M]_D$ similar to that obtained by Graham in case of metallic salts (Perti 1947)

As a further extension of Hädrich's idea it may be suggested that molecular rotation of a salt of an optically active acid with an optically active base should also reach a constant value in dilute solutions which would be the algebraic sum of the rotations of the cation and anion As for example the value in dilute solution of morphine hydrochloride is -371 and of α-bromocamphor π-sulphonic acid is +271 the value for morphine-α-bromocamphor-π-sulphonate is -100° ($-371 + 271 = -100^{\circ}$) (Gilman, 1943)

From the above it is evident that the specific rotation of an ionizable solute when dissolved in an ionizing solvent is dependent upon the degree of dissociation which in turn varies with concentration and temperature It also follows that the phenomenon of association of molecules of the solute influences the specific rotation although direct data on this point is rather meagre Gr-

nez (1864a b) studied the optically active vapours of camphor turpentine essence of orange and essence of bigarade in connection with his experiments on the effect of temperature on optical rotatory power He also noticed that the rotatory power of camphor was identical both in the liquid and vapour state turpentine showed a very small decrease of rotatory power on vapourization a decrease which was more marked in the case of essence of orange and b garade Freundler (1895) studied rotations for pure methyl (+) tartrate and noti ed that for pure substance the value was +2.1 whereas a benzene solution gav a value -8 8° Cryoscopic determinations showed a molecular weight of 411 in benzene whereas the calculated value is 178 Lowry and Gore (Lowry et al 1932) studied the rotatory power of the vapours of camphor and camphorquinone and noticed that these compounds in the vapour state exhibited complex dispersion. Up to this time, however there has been practically no attempt to correlate the optical activity of active liquids to that observed in their vapours.

The influence of organic solvents on rotatory power was also noticed early by Freundler (1894) He found for instance, that the value of rotat on for propylic diacetyl tartrate varies from +36 7° in carbon disulphide to −26° in bromoform whereas the compound itself g ves the value +13 4 In a like manner the value for di-n-valeryl tartrate varies from +8.2° in acetone to −4 7° in bromoform, the compound itself having the rotation +6 7 He suggested that such changes in rotatory power may be due to either polymerisation of the active molecule or combination of the active substance th the solvent.

Moreau (1894) studied the rotatory power of camphor dissolved in a series of solvents and noticed that isomeric modifications of th same solvent show the same rotatory power and further in a homologous series of solvents the rotatory power increases with the molecular weight of the sol ent He was of the opinion that the solvents form true combinations ith camph r esulting in change of rotatory power

It was, however Patterson (1916) ho made an attempt to trace systematically the relation of solvents to active solute He divided substances into two groups viz., electrolytes and non-electrolytes He had noticed earlier (Patterson, 1909) that non-electrolytes like turpentine oil and nicotine are very little effected by the presence of a solvent but in case of ethyl tartrate there is a marked difference in rotation as observed in a nitro naphthalene [ ] = +65°) and ethylene bromide ([a] = −19°) He also noticed (Patterson 1922) that in case of esters of tartaric acid and their derivatives the effect of temperature concentration and solvent is merely to shift the position of the rotation curve In this review Patterson discussed the role of temperature and concentration but did not properly emphasize the rol of the solvent.

The effect of temperature, concentration and solvent is very complicated and it is therefore not surprizing that for nea ly a whole century ince Biot published his classical memoirs n 1832 only empirical results were forthcoming

It is only during 1931 34 that Rule and his co-workers (1931a,b,c 1932a,b,c 1933a,b 1934a,b) attempted to establish definite relationship between dipole moment of the solvent and optical rotatory power of the active solute

Rule and Mitchell (Rule *et al* 1926a,b,c) had observed that the molecular rotations of aliphatic octyl ester in certain aromatic solvents (substituted benzenes) was lower than in benzene. They also noticed that the extent of this change was dependent upon the polarity of the groups attached to benzene. Rule and McLean (Rule *et al* 1931) extended this work by studying optical rotation of methyl ( ) menthyl naphthalate in a series of solvents. It was observed that in solvents of the type C_6H_5—X or CH_3—X the influence of the solvent could be correlated with the polarity of the substituent —X on which depended the dipole moment of the solvent It was also noticed that in majority of cases the rotatory power was found to decrease with the increase in the polarity of the solvent although in certain cases the reverse was observed. The results led to the conclusion that if a comparison is instituted between polar and non polar solvents practically no regularity can be traced. However if the solvents are derived from the same parent hydrocarbon certain regularities are observed The results of Rule and co-workers have been summarized by Lowry (1935) Some of the data of Rule and co-workers is given in the Table below

TABLE 1

Rotatory power of methyl–(–)menthyl naphthalate in different solvents

Solvent	$[M]^{t°}_{5461}$	*Dipole moment* $\mu \times 10^{18}$
C_6H_6	−543°	0
$C_6H_5CH_3$	546	0
C_6H_5I	465	1.25
C_6H_5Br	466	1.50
C_6H_5Cl	463	1.52
$C_6H_5NH_2$	443	1 60
C_6H_5CHO	432	2 75
C_6H_5CN	372	3 85
C_6H_5NO	423	3 89
CH_3COOH	423	0 75 (?)
CH_3OH	383	1 61
CH_3I	336	1 66
CH_3CHO	316	2 71
CH_3CN	23[illegible]	3 0[illegible]
CH_3NO_2	219	3 78
CS_2	437	0
CCl_4	5[illegible]3	0
n C_5H_{12}	[illegible]51	0
n C_6H_{14}	653	0
n-C_7H_{16}	653	0

It is obvious that polar and non polar solvents must be considered separately The influence of the polar solvents is mainly transmitted in the following ways

() *Dipole association* A dipole association may occu between optically active polar solute molecules and the polar molecules of the solvent In such a case the field of force within the active molecule will be weakened and consequently the contribution of the dipolar radical to the total optical activity of the molecule will be reduced It follows that in such cases the more powerful the dipole in the solvent the greater will be the degree of association and greater the observed change in optical rotation. Some of the data of Rule *et al* is given below to illustrate this effect.

TABLE 2

Rotatory power of ethyl—(—) —menthyl naphthalate

Solvent	D pole moment ($\mu \times 10^{18}$)	$[M]^{20}_{5461}$	$[M]_{benzene}$ — $[M]_{solvent}$
C_6H_6	0	$-543°$	———
$C_6H_5Cl + C_6H_6$	1 52	501	4
$o-C_6H_4Cl_2 + C_6H$	2.25	470	73
$p-C_6H\ Cl + C_6H_6$	0	463	80
$C_6H_5NO + C_6H_6$	3 9	527	16
$m-C_6H\ (NO\ ) + C_6H_6$	3 7	510	33
$p-C_6H_4\ (NO_2) + C_6H_6$	0	508	35

This data shows that when disubstituted benzenes are used as solvents the decrease in rotation is nearly double of that observed when a monosubstituted benzene is used It is also obvious that quantitative relations cannot be established because the difference in the highly pola $C_6H\ NO + C_6H_6$ is the minimum.

If we turn our ttention to weakly polar solvents the effects are much less as in this case solvation may lead only to a tendency for th solute d poles to become loosely oriented towards the solute molecules.

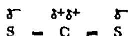
(ii) *Displacement f an optically absorption band* It is known (Scheibe 1926) that the absorption bands of the ketones are displaced more and more towards the ultraviolet as the polarity of th solvent is increased. There is no doubt that such a displacement will effect the partial rotations associated with absorption bands.

From the above considerations it is obvious that the hydrocarbon residue (C_6H_5—or CH_3—) in the polar solute molecules would ha e comparatively a

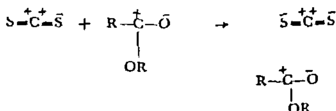
minor influence. This influence will be only to the extent of their effect on the magnitude of the polarity of the polar group. Thus in n-alkyl derivatives of the type R—X the increase in the bulk of R— results in the fall of the frequency with which the polar group —X can come into action and consequently one may expect greater dipole association between solute and solvent in case of, say CH_3 I as compared to C_4H_9 I (Rule, 1924[a,b,c]). The effect of branched chains in screening off the polar group is observed in the case of n— sec— and tert.— butyl chlorides and alcohols (Rule, 1926) or in comparing effects of aldehydes with the corresponding methyl ketones (Rule 1924[a,b,c] 1925[b]

The contrast observed between aryl and aliphatic derivatives is to be sought in the greater tendency of the aromatic nuclei to acquire an induced polarization under the influence of the adjacent or attached polar groups (Rule 1924[a,b,c]). Sometimes the refractive index of the medium may also play a significant role (Rule, 1934[a,b])

A consideration of the non polar solvents indicates sometimes large variations in rotatory power. Non polar solvents like the paraffin hydrocarbons have no permanent dipole and in such cases a minimum of association between solvent and solute can be expected. Rule *et al* (1931[a,b,c]) observed that a maximum constant molecular rotation of methyl-( ) menthyl naphthalate is observed in pentane hexane and heptane. On the other hand there are solvents lik carbondisulphide or carbontetrachloride which have zero moment because of neutralized dipoles. Such molecules, however may exert a definite force on a d pole in its immediate neighbourhood as then the uniform external field in which they have zero moment would no longer be uniform. Take for instance carbondisulphide. It has a neutralized dipole



In the presen e of a polar solute R COOR where R is optically active residue there is possibility of association to a certain extent—



It is an association of this type which has been advanced to explain the fact that m thyl— —,—menthyl naphthalate possesses a molecular rotation of only —437° in carbondisulphide but a rotation of —653° in paraffin hydrocarbons. Similar arguments have been advanced to explain the similarity in the magnitude of rotation observed in p-di-nitrobenzene and mono substituted benzenes

Here it may also be mentioned that the magnitude of the dipole moment is effected by steric hindrance produced by certain groups Thus in case of n— sec.— and tert butyl chlorides, methyl-( ) menthyl naphthalate shows the highest rotation in the case of tert-butyl chloride which has the highest dipole moment (2.14x10[18]) a result which is explained as due to the steric effects of the methyl groups.

When we consider the influence of the solvent on optical rotatory power three types of cases may arise. In one type such as octyl iodoacetate dissolved in pyridine or ( )-menthyl hydrogen naphthalate dissolved in basic solvents, the solvent can form a chemical compound with optically active solute In such cases the influences are bound to be abnormal. On the other hand, the solvent may not enter into chemical combination with the solute but still may have profound influence In case of polar active solute dissolved in polar solvent o a solvent with neutralized dipoles, the dipole association generally would cause a decrease in rotatory power There is, however a third type of case where the solvent does not enter into chemical combination with the active solute nor are the polar influences predominating and yet t may have a significant in fluence on rotatory power An example of this type has been observed by Pribram (1889) who studied 5 % solutions of (+) tartaric acid in water alcohol, alcohol benzene mixture (1 1) alcohol toluene mixture (1 1) and alcohol-chloroform mixture (1 1) The value of $\left[\alpha\right]_{D}^{20^\circ}$ in water was +14 40 in ethanol +3 79° but in a mixture of ethanol and benzene -4 11 in a mixture of ethanol and toluene -6 19° and in a mixture of ethanol and chloroform -8.09° Pertı, Pant and Ghildyal (Perti, et al 1960[a]) have recently observed a profound effect of the solvent on the optical rotatory power of strychnine-o, m- or p-nitro benzoate. Below is given the observed rotatory power of these compounds in pyridine and chloroform.

		$[\alpha]_{5461}$	
		Pyridine	Chloroform
1	Strychnine-o-nitrobenzoate	-85 00°	26 50°
2.	Strychnine-m-nitrobenzoate	94 04°	5.51
3	Strychnine-p-nitrobenzoate	97 00°	0

In the case of strychnine p-nitrobenzoate the zero rotation in chloroform is not due to racemization for if the chloroform is removed and the solute recovered, it shows a specific rotation $[\alpha]_{5461}$ = 97 00° in pyridine. They also noticed that when a mixture of pyridine and chloroform in different proportions is taken as the solvent the observed rotation is between 97 00° and 0° (Perti et al, 1960[b]) In th case observed by Pribram it is obviously possibl to obtain zero rotation in a suitable mixture of solvents.

If the optically active compound has a strong tendency to exist in associa ted states it would apparently give anomalous results. A non polar solvent

or slightly polar solvent if unable to disrupt the association complex even at high dilution would be expected to behave as a highly polar medium. Such effects are probably traceable in the case of ethyl tartrate (Rule 1928) and β-nitro octane (Rule 1927 b c)

There is very little doubt that polar influences play a great part in the effect the solvents produce on rotatory power But there are factors such as association dissociation and solvation whose effect in certain cases cannot be minimised Again all these influences may be complicated by steric factors. The result of this is that the influence of the solvent on rotatory power remains more or less unpredictable Singh *et al* (1944) have noticed that sometimes the order of the magnitude of rotatory power is opposite to the order of the magnitude of dielectric constant of the solvent as expected from a consideration of dipole association as for example in the case of o- m- and p- toluidine salts of Reychler s acid in chloroform pyridine, ethanol, methanol and water Sometimes quite the reverse result is obtained and no order whatsoever is observed as in the case of o- m- and p-chloro phenyl imino-(+) camphor in benzene chloroform, ethyl acetate pyridine, acetone, ethanol and methanol (Singh *et al* 1936)

It is also interesting to note that Buchanan (1938) has found that rotations of optically active compound in hydrogen containing solvents differ from those observed in corresponding deuterium solvents though the magnitude of difference is usually less than one degree

The influence of the solvent on rotatory power must be taken int account in carrying out comparison of optical rotatory power in solution The rotatory power of an active compound in one solvent cannot be reasonably compared with the rotatory power of another compound in another solvent. A reasonable comparison of rotatory power n solution between different active compounds can only be made if the measurement of rotatory power are carried out in the same solvent, at the same temperature using similar concentrations. If this is not done the results are likely to be vitiated by the influence of the solvent

Wave length of light used The dependence of rotatory power on wave length of light used was noticed early by Biot (1052) In a paper read before *Academie des Sciences* on Aug 16 1832 he stated All substances other than the tw tarta ic a ids dextro and laevo in which molecular rotatory power has been recognised upto the present, impress upon the planes of polarization of the simple rays hich compose white light, deviations which are unequal and always ncreasing th tl refrangibility Thus the dispersion of these planes is always of the same character as that which is observed in spectra produced by prismatic refraction

In the case of quartz Biot had found that the rotation varies inversely as the square of the wavelength of light used

$$\alpha = \frac{K}{\lambda^2} \text{ where K is a constant}$$

Tartaric acid, however showed an entirely different type of rotatory dispersion

Biot's inverse square law was later modified by von Lang (1863) who expressed the relation by the formula.

$$\alpha = A + \frac{B}{\lambda^2} \quad \text{where A and B are constants}$$

Boltzmann (1874) criticised the equation given by Biot and von Lang and showed that the dispersion can better be expressed by an expression of the type

$$\alpha = \frac{B}{\lambda^2} + \frac{C}{\lambda^4} + \frac{D}{\lambda^6} + \quad \text{where B C,D are constants.}$$

For all practical purposes, however terms beyond the second may be neglected and the expression can be restricted to

$$\alpha = \frac{B}{\lambda} + \frac{C}{\lambda^4} \quad \text{where B and C are constants.}$$

The first satisfactory equation for the relationship of rotation and wave length of light used was given by Drude (1900) who based his analysis on a consideration of a dissymmetrically isotropic medium A dissymmetrically isotropic medium would result if all the molecules were irregular tetrahedra of the same kind, the tetrahedra of the opposite kind (that which is the image of the first) being altogether wanting The same would be true if one kind existed in smaller numbers than the other A graphical representation may be obtained by conceiving that, because of the molecular structure the paths of the ions are not short straight lines, but short helices twisted in the same direction and whose axes are directed at random in space. In such a medium the rotation of the plane of polarisation is given by the equation —

$$\alpha = \frac{K_1}{\lambda^2 - \lambda_1} + \frac{K_2}{\lambda - \lambda_2} + \frac{K_3}{\lambda^2 - \lambda^2_3} +$$

where K_1 K_2, K_3 are constants depending upon the number of vibrators in unit volume and other constants of the medium and λ λ_2, λ_3 correspond to characteristic frequency of the vibration The equation can conveniently be put as

$$\alpha = \Sigma \frac{Km}{\lambda^2 - \lambda_m}$$

It may be mentioned here that Kuhn (1933) pointed out that the mathematical treatment of Drude is defective the formula that he is valid. In a more convenient form the formula has been given by (1909) and Bruhat (1915a,b)

$$\alpha = \frac{\pi\gamma^2}{c^2} \quad \gamma^2 \frac{\gamma^2 - \nu^2}{(\gamma_0^2 - \gamma^2)^2 + \gamma^2\gamma'^2} \quad D$$

and

$$\phi = \frac{\pi\gamma^2}{c^2} \quad \gamma^2 \frac{\gamma\gamma'}{(\gamma^2 - \gamma^2)^2 + \gamma^2\gamma'^2} \quad D$$

where α = angle of rotation ϕ = the ellipticity in the region of absorption, γ = the head of the absorption band γ' = damping factor c = velocity of light and D = a constant. Outside an absorption band in the region of transparency the term $\gamma\gamma'$ may be neglected the ellipticity is zero and the expression for rotation becomes

$$\alpha = \frac{\pi\gamma_0^2}{c^2} \quad \frac{\gamma^2}{\gamma_0^2 - \gamma^2} \quad D$$

In terms of wavelength (λ) putting K = π D the expression reduces to

$$\alpha = \frac{K}{\lambda^2 - \lambda_0^2}$$

which is the same expression as given by Drude Generally the simple one term Drude's equation is sufficient to express the experimental data. In certain cases, however a two term equation is required The accuracy of the experimental data seldom warrants the use of more than two terms.

It has been pointed earlier that Biot noticed the difference in rotatory dispersion of quartz and tartaric acid Biot divided optically active substances into two classes viz., (i) those which obeyed his inverse square law and (ii) those which did not obey his inverse square law The compounds obeying Biot's law are generally called as exhibiting *normal rotatory dispersion*. Their characteristic, according to Arndtsen (1858) is that the rotation increases with the refrangibility of the rays. Tschugaeff (1914) clearly stated that in normal rotatory dispersion the rotatory power increases progressively with diminishing wave length A more precise definition was given by Lowry (1915 1924) According to this definition in *normal* rotatory dispersion α, $d\alpha/d\lambda$ and $d^2\alpha/d\lambda^2$ must remain constant in sign throughout the range of wavelengths to which the medium is transparent Under these conditions the definition asserts (i) that there must be no *reversal of sign*, which would give $\alpha = 0$ at the point of reversal (ii) that there must be no *maximum* which would give $d\alpha/d\lambda = 0$ and (iii) that there must be no *point of inflexion* or *reversal of curvature* corresponding with $d^2\alpha/d\lambda^2 = 0$ which would make the curve concave as viewed from the axis of wavelength during a part of its course instead of remaining always complex. Lowry 1935)

The unorthodox type of compounds such as tartaric acid (Arecke 18 were said by Landolt 18 7) to exhibit *anomalous rotatory dispersion*. Tartaric acid gives a maximum for rotation in the green a *reversal* of sign in the ultraviolet and the equation of the curve discloses an *inflexion* in the red region if the solution is diluted the maximum is displaced towards the violet and the inflexion

comes in the middle of the visible spectrum, conversly by increasing the concentration (using glassy tartaric acid) the *reversal of sign* comes into blue or green region and the *maximum* is displaced into the red or infra-red region (Lowry 1935). Thus in this case all the three conditions for normal rotatory dispersion are violated simultaneously but in different regions of the spectrum.

Lowry has suggested a simple classification. According to this (Lowry 1914) a rotatory dispersion is said to be *simple* if it can be expressed by a one term of Drude's equation $\alpha = \frac{K}{\lambda^2 - \lambda_0^2}$. The constant K is termed the *rotation constant* and λ_0 the *dispersion constant*. Any dispersion which cannot be expressed by Drude's one term equation is said to be *complex*. This is essentially a practical classification and its main purpose is to distinguish between compounds which conform to the requirements of a one term Drude's equation within the limits of experimental accuracy and which do not conform to this equation, although their dispersion can usually be represented by using two such terms. Theoretically speaking (Lowry 1924 ; Kuhn 1929a-b) the optical rotation of every optically active molecule must necessarily include a long series of partial rotations since the dissymmetry of the molecule must influence all the electrons to a greater or smaller extent. The one or two term Drude's equation represents an ideal approximation within the limits of experimental accuracy. For instance, in those cases where the deviations from simple equation are too minute to be detected readily although the existence of a second term can be inferred in other ways, Lowry recommends the use of the term *pseudo-simple rotatory dispersion* to describe it.

If one compares this practical classification with the classification of rotatory dispersion as normal or anomalous, it can readily be seen that in doubtful cases a decision can be obtained by a process of extrapolation either graphically or mathematically. Graphically the distinction between anomalous and normal dispersion depends on finding out whether the dispersion curve does or does not cut the axis of zero rotation when prolonged into the infra-red. Mathematically this distinction can be decided by calculating a two term equation of the Drude type and comparing the relative magnitudes of the two rotation constants (K_1 and K_2) with those of the corresponding dispersion constants (λ_1, λ_2) for the condition for reversal of sign is that for some value of λ, $\alpha = 0$

$$\frac{K_1}{\lambda^2 - \lambda_1^2} = \frac{K_2}{\lambda_2 - \lambda_2^2} \quad \text{or} \quad \frac{\lambda^2 - \lambda^2}{\lambda^2 - \lambda_2^2} = \frac{K_1}{K_2}$$

or the condition for maximum ($d\,\alpha/d\lambda = 0$) would lead to the relation

$$\frac{\lambda^2 - \lambda_1^2}{\lambda - \lambda_2^2} = \sqrt{\frac{K_1}{K_2}}$$

or the point of inflexion ($d\ \alpha/d\lambda^2 = 0$) would give the relation

$$\frac{\lambda^2 - \lambda_1}{\lambda^2 - \lambda_2^2} = \sqrt[3]{\frac{K_1}{K_2}}$$

A simple graphical test to distinguish between simple and complex rotatory dispersion is to plot $1/\alpha$ against λ^2 (Lowry 1913) as was done by Biot (181? to demonstrate his law of inverse squares in the case of quartz In case of simple dispersion the curve would be a straight line from which a rough value of the dispersion constant can be obtained This preliminary test should, however be followed by an algebraic analysis as this simple graphical test is too inaccurate to serve the purpose of analysing rotatory dispersion (Hunter 1924 Lowry 1924)

It may be mentioned here that Lowry s mathematical criterion for describing rotatory dispersion curves is sufficient for many purposes. It has recently been proposed by Djerassi and Kline (1957) that the rotatory behaviour be characterized by an enumeration of the maxima, minima and inflexions of the rotatory dispersion in cases where a full reproduction of the curves of rotatory dispersion is not possible Their proposal is not in direct conflict with Lowry s but is perhaps more useful in describing succintly a large number of data currently being produced by the help of new and improved instruments now available According to their suggestion a rotatory dispersion curve is said to be *plain* when it does not exhibit a maximum (peak) or a minimum (trough) regardless of the fact whether they can be expressed by one term Drude equation or not In other words the curve is said to be *plain* when it has no maximum or minimum and it is immaterial whether it crosses the zero rotation axis and changes sign at some stage The plain dispersion curves are called *positive* or *negative* depending upon whether they rise or fall toward shorter wavelengths. The curves designated as *anomalous* by Lowry are described as *Cotton Effect Curves* A *positive Cotton effect curve* is one in which the peak occurs at longer wavelengths and when the *trough* occurs at longer wavelengths it is called a *negative Cotton effect curve* The name extremum has been suggested by Djerassi 1960) to cover both *peaks* and *troughs* A third name *multiple Cotton effect curve* has been suggested for more complicated dispersion curves in which two or more *peaks* with a corresponding number of *troughs* occur Kuhn has made the suggestion (Kuhn 1958) that since any further use which can be made of rotatory dispersion is a statement concerning the Cotton effect or anisotropy factor of the absorption bands of the molecules, it would be preferable to give the data in terms of intensities and widths of dichroic bands or else of intensities, widths, and anisotropy factors of the absorption bands measured

It may also be mentioned that Heller has recently (Heller 1958) suggested that a plot of $1/\alpha.\lambda^2$ versus $1/\lambda^2$ is even more significant than the plot of $1/\alpha$ against λ^2 He has also pointed out that the Drude equation in its simple form has been is and will continue to be of major usefulness. Djerassi (1960) has however expressed doubts about the usefulness of Drude equation specially in the fields of peptide and proteins. Upto the present, however there is no better substitute of Drude s equation available and it is continued to be used in a helpful manner

In comparison of rotatory power it is obvious that rotatory dispersion of the compounds under consideration should be analysed first. Comparison would have significant value only if the compounds under consideration exhibit similar type of dispersion in the range under consideration.

References

1. Arndtsen, *A. C. P.*, 54 (III) 409 (1858)
2. Balfe Hargreaves and Kenyon, *Jour. Chem. Soc.* 1861 (1950)
3. Biot, *Mem. Acad. Sci.*, 2, 41 136 (1818) 15, 207 (1838) *Mem. de l'Acad.* 15 93 (1844) *Ann. Chim. Phys.* 10 (3) 385[b] (1844) *A. C. P.* 36 (III) 267 (1852)
4. Boltzmann *Pogg. Ann. Phys. Chem.*, Jubelband, 128 (1874)
5. Born and Jorden, *Elementare Quantenmechanik*, Berlin (1930)
6. Boys *Proc. Roy. Soc.* 144 A, 655-692 (1934)
7. Bruhat, *Ann. de Phys.* 3 (ix) 232 417[b] (1915)
8. Buchanan, *Chem. & Ind.*, 748 (1938)
9. Carrara and Gennara, *Gazetta*, 24 (III) 484 (1894)
10. Cook, *Ber.* 30 294 47 167[b] (1897)
11. Djerassi, *Optical Rotatory Dispersion*, Chap. I (1960)
12. Djerassi and Klyne *Proc. Chem. Soc.* 55 (1957)
13. Drude *Theory of Optics* Leipzig (1900)
14. Frankland and Mac Gregor *Trans. Chem. Soc.*, 760 (1894)
15. Freundler *Compt. rend.*, 556 (1894) *Ann. Chim. Phys.* 4 (7) 256 (1895)
16. Gernez, *Ann. Ecole Normale* 1 1 38 (1864); *Compt. rend.* 58 1108[b] (1864)
17. Gilman, *Org. Chem.*, I, 295 (1943)
18. Graham, *Trans. Chem. Soc.*, 101 746 (1912)
19. Hadrich, *Z. Physik. Chem.*, 12, 476 (1893)
20. Hargreaves, *Jour. Chem. Soc.* 2953 (1953)
21. Heller *J. Phys. Chem.* 62, 1569 (1958)
22. Hunter *Jour. Chem. Soc.* 125 1198 (1924)
23. Kirkwood, *Jour. Chem. Phys.* 20, 561 (1952)
24. Krecke, *Arch. nier.* 7 97 116 (1872)
25. Kuhn, *Ber.* 62, 1727 (1929) *Z. Physik. Chem.*, B 4, 14[b] (1929) 20 B, 325 (1933) *Trans. Farad. Soc.* 26 293 (1930)
26. Landolt, *Ber.*, 6, 1077 (1895); *Ann.* 189, 274 (1877) *Das Optische Drehungsvermogen Organischer Substanzen* Brunswick (1879)
27. Lowry et al, *Trans. Chem. Soc.* 103 1075 (1913) *Trans. F rad. Soc.*, 10, 96 (1914) *T ans. Chem. Soc.* 107 1195 (1915) *Phil. Trans.* 222 (A) 249-308 (1922) *Jour. Chem. Soc.*, 125, 2511 (1924) N ture 113 566 (1924); *Proc. Roy. Soc.* 133 (A) 13 (1932); See also *Optical Rotatory Power* (1935)
28. Moreau, *Chem. Soc. Abs.* ( ) 613 (1894)
29. Natanson, *Jour. de Phys.* 8 (iv) 321 (1909)
30. Oudemans, *Ann.*, 182, 52 (1876)
31. Patterson, *Trans. Chem. Soc.* 79 180 (1901) 81 1097 (1902); 93 355 1849[b] (1908) 97 2118 (1910); 109 1139 (1916) *Proc. Roy. Soc.* 39 18 (1919) *Sci., Progress* 65, 60 (1922)
32. Pertl, *Curr. Sci.*, 16 315 (1947)
33. Pertl, Pant and Ghildyal, *Vij. Par. Anu. Pat.*, 3, 41[b] (1960)
34. Pertl and Pant *Proc. Nat. Acad. Sci.* 29A, 284[b] (1960)
35. Pictet, *Arch. Geneve* 7(3) 82 (1882)

36. Pribram, *Ber* 22 6 (1889)

37 R le *et al*, *Jour Chem. Soc.*, 125 676^{a}, 678^{b} 679^{c} (1924); 127 2653^{a} 2655^{b} (1925) 55?, 1406^{b} 1412^{c} 3202^{d} (1926); 669^{a} 674^{b} 2652^{c} (1931) 1400^{a} 140? 2?? (1932) 376^{a} 1217^{b} (1933) 351^{a} (1934); *Nature* 133^{b} 910 (1934) See also *Optical Rotatory Power* (1935)

38. Scheibe *Ber* 59 2617 (1926)

39. Singh *et al* *Proc Lahore Phil Soc* VI 15 (1944) *Proc Ind. Acad Sci* 22A, 81 263 (1945) *Jour Ind. Chem. Soc.*, 33 821 (1956)

40. Thomas and Jones, *Trans. Chem. Soc* 89 284 (1906)

41 Thomsen, *Ber* 15 441 (1882)

42. Tschugaeff *Trans Farad. Soc.*, 10 70 (1914)

43. Von Lang *Pogg Ann Phys Chem.*, 119 74 (1863)

44 Walden, *Z Physik Chem.*, 15 196 (1894)

PART—II

EFFECT OF NUTRITION ON THE FECUNDITY LONGEVITY AND SEX RATIO OF *BRACON GSECHIAE* ASHMEAD ND *TRICHO GRAMMA EVANESCENS MINUTUM* RILEY USING *COR CYRA CEPHALONICA* STAINTON AS THEIR HOST REARED ON VARIOUS SYNTHETIC* DIETS

PART—II

PRELIMINARY TRIAL OF THE FIELD EXPERIMENT IN THE BIO LOGICAL CONTROL OF MAIZE AND JOWAR STEM BORER *CHILO ZONELLUS* SWINHOE

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PART I

1 The growth of *Corcyra* larvae has been definitely better in the medium of whole wheat than in the media of whole jowar and whole maize.

2. Crushed jowar has supported significantly better growth of *Corcyra* larvae than that supported by the media of crushed wheat and crushed maize.

3 Wheat, maize and jowar in the flour form do not maintain the growth of *Corcyra* larvae better than that maintained by the medium of crushed jowa alone.

In brief the medium of crushed jowar has supported the best growth than the other media. This clearly indicates that the texture of the medium also plays an important role in maintaining the particular standard of growth. That is the reason why the medium of jowar n the flour form supports the worst growth, whereas in the crushed form it supports the best growth in comparison with that supported by the other media.

4 The present studies have amply demonstrated that the mixture of crushed groundnut with crushed jowar has supplied the deficient nutrients of the crushed jowar to the medium which has suported the better growth of *Corcyra* larvae than that on the medium of crushed jowar alone

5 *Corcyra* larvae during their first instar die in the medium of fat free crushed jowa but they continue to grow if they are provided the same di t

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after growing for the period of fifteen days on the medium of crushed jowar. This clearly indicates that either *Corcyra* larvae are able to dispense with fat in the later instars of their life or they are able to meet their requirement for fat by converting other nutrients, like carbohydrates, proteins etc. into fat, or they are able to store up sufficient quantity of fat during the feeding period of the first fifteen days, which is enough to satisfy their demand for fat for the growth of *Corcyra* larvae during their later life.

6 The groundnut fat (the ether extract of crushed groundnut) mixed with crushed jowar does not support the better growth of *Corcyra* larvae than that supported by the crushed jowar whereas the crushed groundnut mixed with crushed jowar does support the better growth of the host larvae than that supported by the crushed jowar. This clearly indicates that the deficient nutrient of crushed jowar which is, though, supplied by the crushed groundnut, is not present in the fatty component of the crushed groundnut.

7 Similarly when the casein is added to the medium of crushed jowar *Corcyra* larvae do not grow better on this mixture than what they grow on the medium of crushed jowar alone. So it is evident that *Corcyra* larvae do not require more protein than that present in the crushed jowar and it is also indicated to some extent that as their requirement for proteins are not much, the protein content of groundnut can not possibly supplement the deficient nutrient of the crushed jowar which is, supplied by the crushed groundnut when mixed with crushed jowar

8 The yeast as well as vitamin B complex accelerate the growth of *Corcyra* larvae to an appreciable extent. As the quantity of yeast or the vitamin B complex is increased there is simultaneous increase in the growth. This is true upto a certain point beyond which the increase in the amount of growth is not directly proportional to the further increase in the quantity of yeast or vitamin B complex mixed with crushed jowar

9 The present studies have clearly indicated that *Corcyra* larvae require carbohydrates and proteins in the ratio ranging from 3 7 to 3 [illegible] respectively for their development

10 The synthetic diet VII supports the growth of the host larvae upto the extent that supported by the crushed jowar only

11 The abstract of the results obtained from the experiment conducted to study the effect of various food media on the performance of *Trichogramma evanescens minutum* Riley have been given in the following table I

TABLE 1

Showing the averages of fecundity longevity sex ratio etc. of *Trichogramma evanescens minutum* Riley using *Corcyra* eggs as its host fed upon various mixtures of diets.

S N	Diets	Averages of					
		Longevity in hours		Fecundity	Rate of ovi position (eggs/hour)	Rate of Reproduction (females/generation)	Sex ratio /
		Female	Male				
1	a.C. jowar+10% groundnut	144	38 8	49 0	0 34	16 4	45 0
	c.C. jowar (control)	170 8	68 8	70 0	0 43	37 7	62 4
2	b.C. jowar+10 % casein	84 0	28 9	41 9	0 50	12 7	41 8
	c C jowar (control)	180 0	76 8	82 8	0 47	41 2	60 0
3	d.C. jowar+8% yeast	311 6	115 2	120 4	0 39	90 0	81 9
	c.C. jowar (control)	192 4	58 0	66 6	0 35	33 7	60 7
4	e.C. jowar+vitamin B	240 8	96 4	101 1	0 43	67 4	74 9
	c.C. jowar (control)	188 0	48 0	73 9	0 38	37 0	58 3
5	f.Synthetic diet VII	71 6	24 8	35 1	0 50	11 1	39 9
	c.C. jowar (control)	196 8	60 4	81 9	0 42	40 6	58 9

A glance at the above table shows that so far as the performance (fecundity longevity sex ratio etc. of *T evanescens minutum* Riley is concerned, the sequence of the various food media tried comes in the following order —

1 Crushed jowar mixed with 8% yeast.

2 Crushed jowar mixed with vitamin B complex.

3 Crushed jowar

4 Crushed jowar mixed with 10% groundnut.

5 Crushed jowar mixed with 10 % casein.

6 Synthetic medium VII

12. Th abstract of the results obtained from the experiments conducted to study the effect of various food media on th performance of *Bracon gelechiae* Ashmead have bee given in the following table.

TABLE 2

Showing the averages of fecundity longevity and sex ratio of *Bracon gelechiae* Ashm. using *Corcyra* larvae as its host fed upon various mixtures of diets.

Diet	Longevity in days		Averages of							
	Female	Male	Fecundity	Egg viability	Larval viability	Pupal viability	Rate of oviposition (eggs/day)	Rate of reproduction (females generation	Sex ratio ( )	[illegible]
a.	18 3	6 4	84 9	56 4	67·6	93 7	5 1	9 5	34 7	3 [illegible]
c.	20 2	9 0	159 8	59 1	70 4	95 1	8 1	28 4	46 3	3 93
b	10 4	7 2	101 5	51 0	61 4	90 1	11 8	10 1	36 0	2 90
c.	20 5	9 5	174 3	59 5	71 1	93 8	8 8	31 8	45 4	3 97
d.	25 4	9 9	219 9	69 1	75 4	97 8	10 5	67 4	69 4	5 43
c.	21 7	10 3	179 8	58 7	69 9	93 3	8 8	30 4	44 1	3 67
e.	24 1	8 0	202 4	57 3	70 2	94 2	8 6	49 5	65 1	4 13
c.	22 3	8 3	168 4	60 1	71 9	92 9	8 2	29 5	44 4	3 73
f.	12 2	5 1	61 3	45 2	60 5	85 3	4 5	3 6	32 0	1 93
c.	19 4	9 2	176 3	57 8	68 9	94 2	9 5	29 8	45 7	3 64

A glance at the above table shows that so far as the performance of *Bracon gelechiae* Ashmead is concerned, the sequence of the various food media tried comes in the same order as given for *Trichogramma evanescens minutum* Riley

Part II

1 *Chilo zonellus* Swinhoe is a serious tissue borer of maize (*Zea mays*) jowar (*Andropogon sorghum*) sugarcane (*Saccharum officinarum*) and padd (*Oryza sativa*) in many regions of the world. It is a very serious pest of maize and jowar the poor man's food, in India Various methods have been devised t control this pest, but the biological basis of control as mentioned earlier appears at the moment one of the economic and practical methods for the control of the pest in the Indian Union The egg and the larval parasites, *Trichogramma evanescens minutum* R ley and *Bracon brevicornis* Wesmael respectively were liberated during the course of the present experimentation in 1957 and 1958 for the control of the pest

2. Th minimum and the maximum population per acre of the host eggs in the treated plots in 1957 were 327 and 14 863 and in 1958 were 651 and 7 155 respectively The minimum and the maximum population of *Trichogramma*

parasites per acre in the same plots during the same period of observations in 1957 were 229 and 13 034 and in 1958 were 196 and 5 129 respectively These figures show that there is almost a similar range between the minimum and maximum populations of the host and the parasite. In other words, when the host population is high, the population of the parasite is also high, but when the host population is low the population of the parasite is also low This is in accordance with the dynamics of the host parasite relationship in nature

3 The liberations of the egg parasite showed the percentage of parasitism as 38 0 and 93 1 and 29 0 and 85 1 during 1957 and 1958 in the control as well as in the treated plots respectively

4 The percentage of parasitism continues to increase in the treated plots upto a certain level beyond which there is no significant increase in the percentage of parasitisation this is the reason why in the sixth observation the percentages of parasitism in the treated plots are 92 4 and 84 6 in 1957 and 1958 respectively whereas in the seventh observation they increased only to 93 1 and 85 1 in 1957 and 1958 respectively The difference in the increase between the percentages of parasitism observed during the sixth and the seventh observations is not significant

5 The host eggs, when parasitised, remains as such without producing adult parasites for a longer period than would elapse if they were unparasitised and produced host larvae. This delay in emergence after parasitisation results in accumulation of parasitism. During the course of the present investigations on account of this accumulation of parasitised eggs in the field, the observed values for parasitism in 1957 are 93 1% and 38 0% in the treated and the control plots respectively The true percentages of parasitism as calculated by the Simmonds (1948) s formula are 89 0 and 27 3 only

6 The above percentages of true parasitism include the greater fraction of replacement of natural mortality of various stages of *Chilo zonellus* Swinhoe The combined calculated value for the mortality due to *Trichogramma* and due to natural factors—physical and biological—is 99 41% in the treated plots, whereas the value for the mortality due to natural factors alone is 96 05% So the difference between the two, that is, 3 36% is the value of mortality due to parasites. This clearly indicates thefraction of the host population fo the destruction of which the liberations of *Trichogramma* were essential.

7 The larval population of *Chilo* increased to the maximum level of 9604 and 10,388 caterpillars per acre in 1957 and 1958 respectively Later on they decreased to the minimum level of 9,243 nd 5,297 caterpillars per acre in the same years respectively This showed that the pest population instead of remaining at a stationary level, has decreased below it during the season.

REFERENCES

Simmonds, F J 1948 Some difficulties in determining by means of field samples the true value of parasitic control. *Bull ent. Res.*, 39 (3) : 435—440.

STUDIES ON ENZYMATIC SYSTEMS*

[PREPARATION OF COMPOUNDS STRUCTURALY RELATED TO VITAMIN B_1 AND THEIR STUDIES ON ENZYMATIC SYSTEMS]

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The present investigations deal with Studies on Enzymatic Systems These investigations have been undertaken with the object to investigate primarily the cocarboxylase activity of thiamine hydrochloride and analogous compounds possessing the essential features of this vitamin with a view to throw further light on the mechanism involved in the cocarboxylase activity of such compounds. For this purpose a number of compounds structurally related to thiamine, homologues and analogues of vitamin B_1 have been prepared and their behaviour on the yeast carboxylase has been studied with the help of Warburg Manometric Method. A separate study on another enzyme system 'Thiaminase' which destroys thiamine has also been made With this object in view the present investigation has been divided into four parts

PART I

Enzymes

In this part a brief introduction to enzymes from biochemical point of view has been given. This includes definition of enzymes, their differentiation from other catalysts, characteristics of enzyme reactions, components of enzyme systems, coenzymes and coenzymes derived from B-vitamins, especially the coenzyme from Vitamin B_1

PART II

Vitamins

Since a number of coenzymes, which have so far been shown to be parts of important enzyme systems, are derived from B-Vitamins, it has been thought to be advantageous to know the functions of vitamins in general and B-Vitamins in particular Therefore, this part includes definition of vitamin as a class and B-Vitamins in particular After this it deals mainly with thiamine—its history of discovery distribution and mode of occurrence physical and chemical properties and different methods of synthesizing it and its homologues (2 R-thiamines) and its analogues (4-substituted) The synthetic routes of Vitamin-B_1 so far used are

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1. Preparation of Vitamin pyrimidine (2 methyl 4 -amino 5 -brom-methyl pyrimidine) and Vitamin thiazole (4 -methyl 5 -b-hydroxy-ethyl thiazole) separately followed by the condensation of the two parts to form thiamine.
2. Building of thiazole nucleus on the pyrimidine part of the vitamin
3. Building the pyrimidine nucleus on the thiazole part of the vitamin.

There are several possible syntheses of the pyrimidine moiety of the vitamin. One synthesis starts with ethyl formate, ethyl 3-ethoxy propionate and acetamidine (Cline and Williams) forming 2 methyl-4 hydroxy-5-ethoxy methyl pyrimidine as the first intermediate. Alternate route of this uses formyl succinate in place of ethyl 3-ethoxy propionate (Andersag and Westphal) and 5 -carboethoxy methyl pyrimidine is the derivative of the pyrimidine which is finally covnerted into vitamin pyrimidine. Another route (Grewe) which is perhaps most useful, is the utilization of 2 methyl-4 amino-5 -cyano-pyrimidine as the first cyclic intermediate which on catalytic reduction is converted into the corresponding 5 -amino-methyl pyrimidine. Starting from 2' methyl-4 -amino 5 -amino-methyl pyrimidine Todd and Bergel built thiazole nucleus by converting the 5 -amino-methyl pyrimidine into the corresponding 5 thioformamido methyl pyrimidine which with r-aceto-r-chloro propyl alcohol is finally converted into the vitamin. A method of building the pyrimidine nucleus on the thiazole part has been described by Andersag.

A number of compounds structurally related to thiamine have been prepared and used in the present studies of the carboxylase activity of the yeast enzym. These compounds may be classified as

1. Compounds with change of substituents in the pyrimidine ring
2. Compounds with change of substituents in the thiazole ring
3. Compounds formed by the replacement of either the pyrimidine or the thiazole ring or both the rings by other ring systems.

Compounds included in class one are of two types (a) change of substituents at 2 position (2 ethyl ? n propyl 2 n butyl 2 -phenyl and 2 -benzyl thiamines) (b) change of subsituents at 4 -positions-4 -hydroxy thiamine 4 methyl amino and 4 -dimethyl amino thiamines.

For the preparation of 2 R thiamines (R = C_2H_5, C_3H_7, C_4H_9, C_6H_5, $C_6H_5CH_2$) the synthetic route I has been adopted. The required amidines (3) were prepared by the method of Pinar from nitriles (1). Ethyl 2' R 4 -hydroxy 5 pyrimidin acetate (5) was prepared by adding eq molecular mixture of pure diethyl succinate and ethyl formate in could to equivalent amount of powdered sodium suspended in absolute ether or sodium dried benzene which formed sodium formyl succinate as a yellow mass. When an alcoholic solution of an amidine was added to the above sodium derivative (5) was obtained in 50-60 yield. By heating (5) with red ... POCl$_3$ or PCl$_5$ in d benzene (6) was obtained which with alcohol ...

gave 2' R-4 -amino-5 -pyrimidine acetamide (7). By Hoffman's degradation the acetamide group at 5 position was converted into the corresponding aminomethyl group giving 2' R-4 -amino-5 -aminomethyl pyrimidines (8). These were also prepared by another route. By action of an amidine on ethoxy methylenemalononitril (9) 2' R-4 amino-5 -cyano pyrimidine was formed. This on catalytic reduction with Raney Ni in alcoholic-ammonia medium at 15-20 atmospheric pressure and at room temperature gave (8).

R—CN —(HCl / C_2H_5OH)→ (2) —(NH_3)→ (3); $CH_2COOC_2H_5$ / $CH_2COOC_2H_5$ + $RCOOC_2H_5$ →

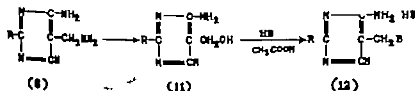
(1) (2) (3)

→ (5) —($POCl_3$ or PCl_5 in benzene)→

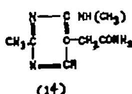
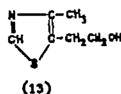
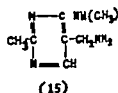
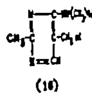
(6) —(NH_3)→ (7) —(Hoffman's degradation)→ (8)

(9) + (10) —(Reduction)→ (8)

When dihydrochloride of (8) was treated with a calculated amount of sodium nitrite solution at 45°–50°C, only the aliphatic amino group was converted into the corresponding alcoholic group forming 2 R-4 -amino-5 -hydroxymethyl pyrimidine (11), which when heated with HB in acetic acid finally gave 2 R-4 -amino-5 -bromomethyl pyrimidine hydrobromide (12).



4-methyl-5-b-hydroxyethyl thiazole (13) was obtained by the sulphite cleavage of thiamine by Williams method. By heating (12) with excess of (13) at 115°–120° 2 -thiamines were obtained. (R = C_2H_5, C_3H_7, C_4H_9, C_6H_5, $-C_6H_4CH_3$)

(14) (13) (15) (16)

(b) Three anologues of thiamine namely 4-hydroxy thiamine 4-methyl-amino thiamine and 4-dimethyl amino thiamine have been prepared. Oxy-thiamine was prepared by the action of nitrous acid on the vitamin in cold. 4-dimethyl-amino thiamine was prepared by heating the vitamin with formaldehyde and formic acid. For the preparation of 4 methyl amino thiamine, ethyl 2'-methyl 4-chloro-5 pyrimidine acetate (6) ($R = CH_3$) was heated with 30% methyl-amine under pressure to yield 2-methyl 4-amino 5 pyrimidine acetamide (14) This on Hofmann degradation was converted into the corresponding 5-aminomethyl pyrimidine (15) This on heating with sodium nitrite solution at 40°-45°C in concentrated hydrochloric acid medium was converted directly into (16) which with thiamine thiazole gave 4-methyl amino thiamine.

Other compounds belonging to class 2 and 3, which have been prepared are as follows —

1 2 methyl 4-amino 5-(2-amino 4-methyl thiazolium chloride) methyl pyrimidine hydrochloride.
2 3-N benzyl 4-methyl 5 b-hydroxy ethyl thiazolium chloride.
3 3-(2-hydroxy 5 nitro) benzyl 4-methyl 5-b-hydroxy ethyl thiazolium chloride.
4 4 or 5-N (4-methyl 5-b-hydroxy-ethyl thiazolium chloride) methyl iminazole hydrochloride
5 2 methyl 4-amino 5 (2 methyl pyridinium chloride) methyl pyrimidine hydrochloride
6 2 methyl 4-amino 5 (3-methyl pyridinium chloride) methyl pyrimidine hydrochloride
7 2' methyl 4 amino 5-(4-methyl pyridinium chloride) methyl pyrimidine hydrochloride.
8 2' methyl 4-amino 5-(4 or 5- hydroxy methyl iminazolium chloride) methyl pyrimidine hydrochloride
9 2 amino 3-benzyl 4 methyl thiazolium chloride
10 N benzyl 2 methyl pyridinium chloride
11 N benzyl 3-methyl pyridinium chloride.
12 N benzyl 4-methyl pyridinium chloride
13 4 or 5-N (2 amino 4 methyl thiazolium chloride) methyl iminazole hydrochloride

Cocarboxylase has been prepared by heating thiamine hydrochloride in a solution of phosphorus-pentoxide in 85% phosphoric acid. The details of its separation from unreacted thiamine and its purification through phospho-tungstate are given

Part III

This part deals with physiology of thiamine. A brief report on the symptoms developed in animals including man, in thiamine deficiency states the enzymatic functions of thiamine specificity of thiamine molecule and the effects of compounds structurally related to thiamine as reported by previous workers has been given

The effect of homologues, analoges and other compounds described in Part II on the cocarboxylase activity of yeast enzyme has been studied manometerically The yeast apoenzyme needed for this work was prepared from dry Bakers yeast by washing it successively with 0 1 M acid potassium phosphate, water and 0 1 disodium hydrogen phosphate and finally with water All the studies were carried out at pH 6 2, at which the maximum activity of the reconstituted enzyme (apoenzyme plus a given concentration of cocarboxylase and Mg ions) has been reported by other workers. Such a preparation, although not completely free from the coenzyme was found good enough for undertaking the study of the effects of the prepared compounds as the amount of carbon dioxide evolved by the apoenzyme alone from the decarboxylation of pyruvate was small. This kind of study includes

1 *Effect of Cocarboxylase*—Effect of different concentrations of cocarboxylase (from 1 to 20 micro-grams) on the carboxylase activity of washed yeast enzymes has been studied in presence of 100 micro-grams of magnesium ions.

2. *Effect of Metallic Ions*—When apoenzyme preparation corresponding to 0 1 gram of dry yeast (was used along with 1 micro-gram of cocarboxylase, (a) maximum enzymatic activity was observed with 100 micro-grams of magnesium ions. A comparative study on the effects of Mn Zn and Mg ions was also undertaken and it was found that the relative effectiveness of manganese ions is considerably greater than magnesium ions and zinc ions were found to be nearly half effective. The relative activity of Zn and Mn ions as compared with the activity of Mg ions are as follows —

Mn^{++} 276% Mg^{++} 100% Zn^{++} 53 6%

3 *Effect of Thiamine*—It has been observed that when different concentrations of thiamine are added to apoenzyme (corresponding to 0 1 gram of dry yeast) in presence of 100 micro-grams of Mg ions, before adding 1 micro-gram of cocarboxylase, thiamine shows an activation effect upto a concentration level of 8-12 micro-grams, after which it shows a tendency to inhibit th cocarboxylase activity This action of thiamine is not due to ts conversion to additional amount of thiamine-coenzyme, but it is presumably due to its inhibitory action on other enzymes, which inactivate the coenzyme. Percentage of inhibition of such enzymes by thiamine at different concentration levels has been calculated on the assumption that a given amount of activation corresponds to an equal amount of inhibition of the dephosphprylation of coenzyme The values are

Concentration of Thiamine $M \times 10^{-8}$	% inhibition
0 33	20
0 66	38.5
1 335	62 5
2 67	100 0

4 *Effects of Pyrimidines*—Effect of two types of pyrimidine derivatives *i. e.*, (a) Ethyl 2 R 4 -hydroxy 5 -pyrimidine acetate and (b) 2' R 4 -amino 5 -amino methyl pyrimidine (R = C_2H_5 C_3H_7 C_4H_9-, C_6H_5- and -$CH_2C_6H_5$) has been studied It has been found that pyrimidines having hydroxyl group are without any effect. In the case of (b) pyrimidines (R = CH_3, C_2H_5 C_3H_7 C_4H_9 C_6H_5 $C_6H_5CH_2$) the activity decreases with the ascending order of alkyl groups at 2 position 2 methyl 4 amino pyrimidine is most effective. This activity is maximum at 120 micro-grams (obtained by extrapolating the graph) for 2' methyl derivative In the case of 2'-ethyl derivative activation increases upto a concentration level of 50 micro-grams and for 2'-propyl derivative it comes to 25 micro-grams. 2 -n-butyl-4 amino-5 -amino pyrimidine does not show any activation effect but only inhibitory effect. 2' pheny pyrimidine is without effect while the corresponding 2'-benzyl pyrimidine shows a slight inhibition

The compounds, which have been found ineffective are as follows—

1 3 N Benzyl-4 methyl 5-b-hydroxyethyl thiazolium chloride
2 3-N-(2 hydroxy 5-nitro) benzyl-4-methyl 5-b-hydroxyethyl thiazolium chloride.
3 4 or 5 N (4-methyl 5-b-hydroxyethyl thiazolium chloride) methyl imidazole hydrochloride.
4 4 or 5 N 2-amino-4-methyl thiazolium chloride) methyl imidazole hydrochloride
5 2-amino-3-N benzyl-4-methyl thiazolium chloride
6 N benzyl 2 methyl pyridinium chloride.
7 N benzyl 3-methyl pyridinium chloride.
8 N benzyl 4-methyl pyridinium chloride

The compounds which have been found to show an activation effect, are
1 2 methyl 4 -amino-5 (2-methyl pyridinium chloride) methyl pyrimidine HCl
2. 2'-methyl 4 amino-5 3-methyl pyridinium chloride) methyl pyrimidine HCl
3 2 methyl 4 -amino-5 (4-methyl pyridinium chloride) methyl pyrimidine Hydrochloride
4 2 methyl 4 -amino 5 (2-amino 4-methyl thiazolium chloride) methyl pyrimidine hydrochloride
5 2 -methyl 4 -amino 5 (4 or 5 hydroxy methyl imidazolium chloride) methyl pyrimidine hydrochloride

The activation effect of these compounds is not due to the ability of the whole molecule but it is due to the vitamin pyrimidine made available from the cleavage of these compounds by the enzyme systems As such the activation effect is not the direct activation of cocarboxylase but is indirectly obtained by virtue of their inhibition of dephosphorylation of the coenzyme through the vitamin pyrimidine The percentage inhibition of these compounds at con centration levels of 50 and 100 micro-grams are as follows

Compound	% inhibition at	
	50 μg	100 μg
1	22.5	32 1
2.	32.8	52.6
3	25 0	40 0
4	20 0	31 0
5	12.1	21 4

4 hydroxy thiamine, 4 methylamino thiamine and 4 -dimethylamino thiamine have been found, more or less, without any marked effect on the co-carboxylase activity

A graded activity in the apparent activation" of 2 R thiamines (R = CH C_3H_7 = C_2H_5) in decreasing order has been found but the concentrations at which these show maximum activation (meaning inhibition of the dephosphory lation) of coenzyme are found to be in the reverse order The values of these concentration levels for different 2' R thiamines are 8 micro-grams for thiamine (in presence of 1 μg of cocarboxylase) 10 micro-grams for 2 -ethyl thiamine (in presence of 2 micro-grams of coenzymes) and 6 micro-grams for 2 n-propyl thiamine (in presence of 2 micro-grams of the coenzyme) Above these con centration levels these compounds show inhibitory effect. 2'-n butyl thiamine shows inhibitory effect from the beginning 2' phenyl and 2'-benzyl thiamines do not show any action on the coca boxylase activity The inhibitory effect of 2'-ethyl, 2'-n propyl, 2' n-butyl at higher concentration levels has been ex plained by assuming that these compounds compete with the coenzyme for their union with the apoenzyme through 4 -amino group which is common to both

The conclusions drawn from the present investigations are

1 For inhibition of dephosphorylation of the coenzyme by other inactivating enzyme present in the crude preparation, 4 -amino group in the pyrimidine ring is essential.

2 The magnitude of inhibition is influenced by other substituents in the pyrimidine ring

3 For greater inhibitory effect, a lower alkyl group at 2 position of the pyrimidine ring is desired.

4 At higher concentrations thiamin and its alkyl homologues compete with the coenzyme and thus inhibit the cocarboxylase activity

Concentration of Thiamine $M \times 10^{-4}$	% inhibition
0 33	20
0 66	38.5
1 335	62.5
2 67	100 0

4 *Effects of Pyrimidines*—Effect of two types of pyrimidine derivatives i e, (a) Ethyl 2 R 4-hydroxy 5 pyrimidine acetate and (b) 2' R 4'-amino-5'-amino methyl pyrimidine (R = C_2H_5, C_3H_7, C_4H_9, C_6H_5 and $-CH_2C_6H_5$) has been studied. It has been found that pyrimidines having hydroxyl group are without any effect. In the case of (b) pyrimidines (R = CH_3, C_2H_5, C_3H_7, C_4H_9, C_6H_5, $C_6H_5CH_2$) the activity decreases with the ascending order of alkyl groups at 2 position 2 methyl 4 amino pyrimidine is most effective This activity is maximum at 120 micro-grams (obtained by extrapolating the graph) for 2' methyl derivative In the case of 2'-ethyl derivative, activation increases upto a concentration level of 50 micro-grams and for n-propyl derivative it comes to 25 micro-grams. 2'-n-butyl-4-amino-5-amino pyrimidine does not show any activation effect but only inhibitory effect. 2' phenyl pyrimidine is without effect while the corresponding 2'-benzyl pyrimidine shows a slight inhibition

The compounds which have been found ineffective are as follows —

1 3-N Benzyl-4-methyl 5-b-hydroxyethyl thiazolium chlorid

2 3-N (2-hydroxy 5-nitro) benzyl-4-methyl-5-b-hydroxyethyl thiazolium chloride

3 4 or 5 N-(4 methyl 5-b-hydroxyethyl thiazolium chloride) methyl iminazole hydrochloride.

4 4 or 5 N 2-amino-4-methyl thiazolium chloride) methyl iminazole hyd ochloride

5 2-amino-3-N benzyl-4-methyl thiazolium chloride

6. N benzyl methyl pyridinium chloride.

7 N benzyl 3-methyl pyridinium chloride.

8 N benzyl-4-methyl pyridinium chloride

The compound which have been found to show an activation effect are

1 2-methyl-4-amino-5 (2 methyl pyridinium chloride) methyl pyrimidine HCl

2 2 methyl 4 amino-5 (3 methyl pyridinium chlorid) methyl pyrimidine HCl

3 2 methyl-4-amino-5 (4 methyl pyridinium chlorid methyl pyrimidine Hydrochloride

4 2 methyl 4 amino 5 amino 4-methyl thiazolium chloride) methyl pyrimidine hydrochloride

5 2-methyl 4-amino 5 4 or 5-hydroxy methyl iminazolium methyl pyrimidine hydrochloride

LIGHT ABSORPTION IN PARAMAGNETIC IONS IN SOLID AND STATE OF SOLUTION

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The absorption spectra of the salts of the iron group of metals in crystalline state or in state of solution are quite different from the gaseous atomic spectra. Gaseous spectra do not tell us any thing about the interatomic forces while the absorption bands as obtained in state of solution or in state of crystal are intimately associated with the binding of the metal ion and hence a spectroscopic study on these complexes in crystalline state and state of solution raises the hope of providing us with informations about the crystalline forces.

Of late the results of magnetic susceptibility measurements on single crystals of rare earths and iron group of metals and those of paramagnetic resonance experiments provide a great stimulus to the understanding of the effects of crystalline fields on the energy levels of these ions The iron group of metal ions with an incomplete d-shell have a large number of energy levels in the optical region hence many of the same substances are of interest from optical, magnetic susceptibility and paramagnetic resonance point of view

Paramagnetic resonance techniques can give us information only about energy levels for which the energy separation is of the order of 0 1 cm^{-1} while optical absorption spectra can provide information for energy separation both low and high. The splitting of the lowest energy levels is often related to the nature and spacing of the excited states and hence all the techniques (magnetic susceptibility measurements, paramagnetic resonance and optical absorption) may yield inter-related results Consequently studies on absorption spectra of solids and of solutions are eminently suitable for getting information about the energy levels of the ions in a crystal and that alternatively the ions may be regarded as probes measuring the crystalline electric field and have therefore engaged the attention of a large number of investigators.

As is well known in the iron group of metal ions, M^{++} and M^{+++} all the inner levels are already full. The 3d-shell of the ions, which are getting progressively filled up are the outermost ones, since those occupying the 4s-shell in the free atom have been removed in the f rmation of the ion in the crystal. Hence in the case of the iron group of complex ions the d-electrons are exposed directly to strong and generally asymmetric electric fields in the crystals. As a result the electrons of a given metal ion in solids or in liquids may be regarded

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as under the influence of an asymmetric and strong electric field which splits the energy levels of the ions This problem of stark splitting of energy levels of ions under the influence of crystalline electric fields of different symmetry has been worked out by Bethe (Ann Physik, 3 133 1929 Z Physik, 60 218, 1930 Van Vleck (The Theory of Electric and Magnetic Susceptibilities, Oxford Press, 1932) Penney and Schlapp (Phys. Rev 42 666 1932) The number of Stark levels and their relative separations will depend on the strength and symmetry of the crystal field

From the point of view of the influence of the crystal fields on the energy levels of the ions, the environmental conditions in the neighbourhood of these ions can be broadly divided into three classes.

(*a*) Conditions typified by the rare-earth ions in which the influence of the fields on the energy levels is greatly reduced by the shielding action of the outer shells of electrons, so in the study of the electronic structure of these ions we may treat each (L-S) multiplet separately and consider the splittings of the multiplets due to the crystal field

(*b*) Condition typified by those occurring in the salts of iron group of metals. In this case 3d-electrons are the outer most and hence the electric field effect will be much more pronounced As a matter of fact the effect of field is *strong* enough to break L-S couplings, so that the quantum number J is no more a good quantum number

(*c*) Conditions as typical of the covalent complexes Here the electric fields involved are much larger and their effects on the energy levels are much more drastic In this case even the spin quantum number S is not a good one in other words even the coupling between the spin moments of the various electrons in the 3d-shell, is broken by the electric field and each electron therefore behaves as though it were a 3d-electron and independent of other electrons except for the restriction imposed by Pauli's principle as to the occupation number

The theoretical technique to be employed in the investigation of the energy levels of these ions with three different environmental conditions will naturally be different

Splitting of the energy levels of the ions like Cr^{++} V^{++} F^{++} Fe_{+++} and Mn^{++} in relation to their magnetic behaviour has been subjected to extensive studies by Krishnan and Mookherji (Phys. Rev 50 [illegible] Loc. cit 54, 533 841 1938) Mookherji (Ind Jour Phys, 29 [illegible] 1945 [illegible] (Ind. Jour Phys 22 74 195 276, 1948) and Bose et al (Ph [illegible] R [illegible] 239 163 1937 Loc [illegible] 248 163 19[illegible]) Their measurements have [illegible] influence of a strong and asymmetric crystalline electric field [illegible] of an axially distorted octahedral cluster of water dipoles surrounding the metal ions The crystal field referred above are in general predominant

cubic on which are superimposed comparatively small non-cubic ones Paramagnetic resonance technique as applied to various cupric salts (Abragam and Pryce, Proc. Roy Soc. **206** 165 173 1951 **205** 135 1951 Proc Phys. Soc **63** 409 1950 Bauggley and Griffiths Proc. Roy Soc **201** 366 1950 Bleaney Penrose & Plumpton, Proc. Roy Soc **198** 406 1949 Abe Ono, Hayashi, Shimada and Iwanaga, J Phys Soc. Japan 9 814 1954 Trenam Proc Roy Soc., **66** 118, 1953 Abe Phys Rev., **92**, 1572 1953 Okamura and Date Phys Rev., **92**, 314 1954 and Kozyrev Acad Sciec Of the USSR, Physical series No. 6 21 828-832, 1957) Ni++ (Griffiths and Owen Proc Roy Soc., **215** 451 1952) Co++(Abragam & Pryce Proc Roy Soc., **206** 173 1951) shows the splitting due to the cubic field may be taken to be of the order of 10^4 cm^{-1} and that due to the noncubic field to be of the order of 10^2cm^{-1} Hence transitions between the levels so split will produce absorption spectra lying between ultra violet and infra-red regions and as such will be capable of optical verification

In the state of solution of a given salt the lattice structure breaks down completely while the anisotropic ionic clusters retain their identities (Krishnan Nature, **143** 600 1939 Chakrawarty Sc Cult 7 140 1942) Since they are oriented at random the medium will show no anisotropy of susceptibility but optical absorption should reveal the fine structure in the Stark pattern from anisotropic field splittings

The experimental observations on the absorption spectra on ions in crystals and in solution like Cr+++ Co++ Ni++ and Cu++ by Dreisch and Trommer (Z. Phys. Chem **B45** 37 1939 *ibid* **B45** 19 1940) Jorgensen (Acta Chemica Scadinavica, **9** 1362, 1955) Kiss et al Z anorg allg Chem **245** 356 1941) were theoretically treated by Tanabe and Sugano Jour Phys Soc Japan **9** 753 1954), and by Orgel (J Chem. Phys. **23** 1958, 1955 who found that the number of levels and their relative separations are in accord closely with that what one should expect to occur *in a cubic field* One direct consequence of such a cubic field would be a complete magnetic isotropy for the crystal and any observed deviation from magnetic isotropy will give us some idea of the non-cubic part of the field The extensive magnetic measurements on single crystals containing the above mentioned ions by Krishnan et al Phil Trans **238**, 125 1938) Bose et al (Proc Roy Soc **239** 165 1957 *ibid*. **248** 153 1959) and Bose Ind Jour Phys **22** 74 195 2 6, 1948) show that magnetic anisotropy for Cu++ is 35 Ni++ is 6 Co++ is 76 and C +++ is 4 % and hence anisotropy is not small Moreover systems with perfect octahedral distribution cannot be a stable one Jahn Teller Proc Roy Soc **161** 220 1937 *ibid* **164**, 11 1938) consequently there should be a departure from octahedral symmetry but absorption bands arising from the non-cubic part of the field were not experimentally observed Dreisch and Trommer) It has been pointed out by Van Vleck (J Chem. Phys. **7** 72 1939) and Bose et al

(Proc Roy Soc 248 153 1959) that in the crystalline salts of iron group elements the electric field arises from the cluster of charges immediately surrounding the metal ion (Jahn Teller effect) and the direct and induced effects of charges outside the cluster The direct and induced effect which is essentially of long range character should vary appreciably when the crystals of these ions go in solution and also from salt to salt with same metal ion

The crystalline electric field theory as discussed earlier ascribes a passive role to the ligands. Owen (Proc Roy Soc 227 183 1955) in order to rationalize the fact that the spin-orbit coupling constants in free ions are larger than the values they have in crystals had to picture that there is a charge transfer between the central ion and the ligands, which reduces the orbital contribution by a factor known as the covalency factor the knowledge of which enables one to distinguish between a purely ionic complex and a complex with weak covalent bonding

Thus the study of the absorption spectra of single crystals containing Cu^{++} Ni^{++} Cr^{+++} and Co^{++} ions etc and of their solutions are very interesting from various points of view

The light absorption from 3900°A to 10 000°A for iron group of salts, about twenty in each case like the single sulphate double sulphates of NH_4 K, Rb, Tl, single selenates and double selenates of NH_4 and K single and double halides, single and double nitrates, acetate formate propionate and ammonium salts containing Cu^{++} Ni^{++} Co^{++} and Cr^{+++} ions were studied in different solvents like water alcohols etc at room temperature by using a UVISPEK spectrophotometer

All the chemicals used for the thesis work are of Merks analar y [illegible]

Single crystals of some double sulphates containing the above mentioned ions were also studied to record a systematic account of the consequences of the crystal field on these metal ions. The results so obtained are discussed in the light of the findings from magnetic susceptibility measurements and paramagnetic resonance experiments

Among the various results of interest obtained in these salts may be mentioned the following

1 [illegible] observed absorption bands of the complex [illegible] the forbidden [illegible] between the energy levels of $3d^n$-configuration [illegible] In crystalline [illegible] field arising out of axially distorted octahedral [illegible] of water [illegible] the metal ions and the magnetic [illegible] of their [illegible] dipole transitions coupled with vibrations [illegible] should expe[illegible] of separation of these bands will be [illegible] mined by the [illegible] field and the anisotropic part will have [illegible] influence [illegible] satisfactorily Taking for example [illegible]

salts it is found that cubic splitting $\Delta E \approx 12\,000$ cm^{-1} while tetragonal splitting ΔT is ≈ 500 cm^{-1} and in nickel salts $\Delta E \approx 15\,000$ cm^{-1} $\Delta T \approx 1500$ cm^{-1}

(2) From the energy of separation of these bands, one can evaluate the cubic field coefficient K, which determines the size of the water clusters about the metal ions. It is observed that complexes whose ligands are NH_3 or organic bases have K values larger than those complexes for which the ligands are water dipoles. As an illustration we may cite the case of cupric sulphate whose K $\approx$ 26,000 cm^{-1} while in cupric amino-salts K $\approx$ 34 000 cm^{-1}

Judging the values of K one can say that the crystal field is observed to be stronger in triply ionised complexes like Cr^{+++} than those doubly ionised ions like Cu^{++} Ni^{++} and Co^{++}

(3) It is observed that generally K values for Cr^{+++} and Co^{++} ions in aqueous solution are larger than those in organic solvents. With Cu^{++} and Ni^{++} ions the case is different. Here K values from aqueous solutions are almost equal to those for organic solvents, excepting for pyridine solution in which case K values are larger than aqueous solution values.

(4) For Ni^{++} Cr^{+++} and Co^{++} ion complexes, where F terms lie lowest, the term separation an important spectroscopic constant is calculated from the observed absorption measurements. If salts containing these ions are *purely ionic* then the term separation values in crystals or in solution containing these ions should be the same as for the *free ion*. Our observations show a reduced term separation in Ni^{++} and Cr^{+++} ion salts. This suggests a weak covalent bonding in these salts arising from the partial overlap with 3d-orbitals i.e., with σ- and π- orbitals of the surrounding atoms while in cobalt salts this orbital overlap is negligible

(5) This weak σ- and π- orbital overlap introduces a covalency factor β^2 in the term separation value E for the crystal and hence a comparison with the free ion term separation value E one can evaluate β^2. It is observed that in ordinary ionic salts containing Cu^{++} Ni^{++} and Cr^{+++} ions β^2 values are of the order of 0 85 0.9 0 72 respectively

(6) As mentioned earlier crystal field acting on the metal ions influences their energy levels and ultimately their magnetic behaviour. Hence from magnetic susceptibility measurements or from paramagnetic resonance data one can evaluate these energy levels. These evaluated energy levels do not agree with those observed experimentally since those from susceptibility data or from paramagnetic resonance data contain the covalency factor β^2 within them. Hence β^2-values as calculated from term separation was utilised to deduce mean magnetic moment values and the spectroscopic splitting factor g. It is

observed that the calculated values agree favourably with these measured directly in state of solution

(7) Primarily it may be expected that this covalency factor should not be different for all the salts in which the ions are similarly coordinated with six water dipoles, but there might be an appreciable change in this factor from salt to salt due to the effect of the distant atoms outside the primary cluster. In state of solution the distant atom effects are negligible hence covalency factor should not vary appreciably from salt to salt having the same ion. This is what is observed

(8) On chemical grounds amino-salts and salts with organic bases may be expected to have stronger bonding than the Tutton-salts. Thus we find that in amino-salts acetate and in propionate the covalency factor is made up of two factors arising from σ- and π-orbital overlap while in Tutton-salts π-orbital overlap may be neglected.

(9) Due to larger charge on Cr^{+++} ions than on the ions Cu^{++}, Ni^{++} and Co^{++} the electrons of oxygens of chromic salts have got a tendency to move into the central Cr^{+++} ion in order to even out the charge distribution. As a result π-orbital overlap may not be negligible in case of chromic salts. This is what is observed. In chromic sulphate f^2 is made up of $f^2\pi = 0.9$ and $f^2\sigma = 0.8$ making $f^2 = 0.72$

(10) Excellent agreement with measured values of magnetic susceptibility anisotropy was obtained by attributing the bands at nearly 1[illegible]000 cm^{-1} and at 12,500 cm^{-1} for cupric salts to the splitting by tetragonal part of the crystal field

Similar agreement was obtained in nickel salts by attributing the bands at 13,[illegible]00 cm^{-1} and 15,[illegible]00 cm^{-1} to tetragonal splitting

(11) Th[illegible] incidentally supplies an experimental evidence of the existence of a strong tendency of coordination linkage in iron group of metals causing [illegible] dipoles to form an anisotropic cluster about the metal ions

Th[illegible] [illegible] about Cu^{++} ions were found to be drawn out one[illegible] about Ni^{++} [illegible] th[illegible] w[illegible] found to be compressed one. These are in complete agreement with X-ray and other findings.

(12) Th[illegible] [illegible] magnetic moment values of cupric ions do not [illegible] appreciably from salt to salt whether they are in crystalline state or in aqueous solution [illegible] th[illegible] d[illegible] ta[illegible] t at[illegible] m [illegible] ffects are not very sen[illegible] [illegible] m case of cupric salt [illegible] λ K [illegible] [illegible] calculated from optical measurements on [illegible] crystal and [illegible] λ K [illegible] [illegible] ed from susceptibility measurements on single crystal point to the same [illegible]

In case of nickel salts it is observed that the contribution of the distant atoms to the anisotropy of the water cluster is much pronounced in double sulphates of K, Rb, single sulphate and acetate while in double sulphates and selenates of NH_4 and Tl it is not so pronounced

STUDIES ON THE MORPHOLOGY BIONOMICS AND CONTROL OF SOME INDIAN AGROMYZIDAE

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The genus Agromyza is of international importance and its species occur on all continents. They are injurious to crops of economic importance, specially the Legumes. However the species differ in biology host plants feeding habits, and the character of the injury done to their host plants from place to place. The following four species of family Agromyzidae are known to be widely distributed in India viz., *Melanagromyza phaseoli* Coq (Pea stem borer) *Agromyza obtusa* Mall. (Tur pod fly) *Phytomyza atricornis* Meigen (Pea leaf miner) and *Ophiomyia lantanae* Frog (Lantana seed fly)

Melanagromyza phaseoli Coq is commonly known as the Pea stem Agromyza in India and the 'Bean fly' abroad. It is widely distributed in the far eastern countries. In India this pest has so far been recorded by Fletcher (1914) in Coimbatore and Tennevelly and Ghosh and P Sen (1917) in Bihar and Bengal.

Pea is the favourite food plant of *Melanagromyza phaseoli* Coq., which forms an important item in the diet of large number of people in India. Pea crop in this province is mainly grown for grain green pods to be used as green vegetable and to some extent for fodder The green peas are canned and refrigerated in large quantities It clearly shows that the pea is an economically important crop

The information regarding the Morphology and Bionomics of genus Agromyza is fragmentary and not duly publicised. It was, therefore, considered necessary to work out the morphology of immature and adult forms of *Agromyza obtusa* Mall. and biology of *Melanagromyza phaseoli* Coq in detail which are the important pests in U P

In addition to the external and internal morphology of maggot and adult fly the bionomics of the insect was studied in detail, the knowledge of which can be used in working out the practicable control measures.

The larva of *Agromyza obtusa* Mall. is amphipneustic and has no true head body consists of twelve distinct segments first three thoracic (pro meso and meta) and the remaining nine abdominal. There is no superficial difference between thoracic and abdominal segments. The first body segment bears

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the anterior spiracles and the last segment carries the posterior spiracles and the anus. Each abdominal segment of the body bears a transverse furrow which sub-divides *it into two* and therefore to all appearance one notes double the actual number of segments.

The head appears as *bilobed* when viewed from above. There are two oral hooks which lie in the lateral pouches of the oral cavity The maggot applies them for tunneling through the seeds for breaking down the cotyledons in order to liberate the juice, which forms its food.

Just after the head, lies the thoracic region and abdomen which consists of three segments viz pro meso and metathorax. The segments are marked by circular swellings The first thoracic segment has a pair of anterior spiracles situated near its *posterior* border The last body segment is larger and bears the posterior *spiracles*. The anus is situated on the rear part of the ventral surface between a *pair of lobes*

The alimentary canal is divisible into fore gut, mid gut and hind gut. The *fore* gut consists of mouth, pharynx, oesophagus and globular proventriculus. The common salivary duct opens into the pharynx. The mid gut or ventriculus is *simple* tube and forms most part of the alimentary canal. There are *four bulbory* gastric caecae at the anterior end of the mid gut. The hind gut consists of ileum, colon and rectum which opens at the anus. There are four malpighian tubules which arise in pairs on each side of the gut from a common tube

The nervous system consists of a brain which has two lobes and a compound ganglion The nerve trunks arise from the compound ganglion to various parts of the body

The tracheal system consists of two dorsal trunks extending between the anterior and posterior pairs of spiracles a dorsal commissure in each segment connects the main trunks. The first and last commissures are more conspicuous than others. The viscera and body wall get *trachae from* lateral branches of dorsal *trunks* The lateral branches are connected by a series of lateral *trunks* except the first three

The head of adult *Agromyza obtusa* Mall. is highly modified. It is anteriorly convex in front, the posterior surface being almost flat and slightly concave The genae consist of large compound eye which cover almost the whole of the antero-lateral regions of the head The epicranium on the posterior surface is flat and on the anterior surface it is convex. It contains three ocelli situated on a slightly raised ocellar triangle The antennae arise from the lower end of the frons Antenna consists of three segments scape pedicel and flagellum. Flagellum bears the arista Arista tapers towards the apex and is longer than

whole of the antenna. At the anterior region of the face there are two fronto-orbital bristles tapering towards mouth.

The proboscis consists of two parts, the proximal rostrum and a distal half of the proboscis proper which bears the oral lobes. The sides of the haustellum are formed by the overlapping labrum-epipharynx and labium hypopharynx. The two oral lobes are normally connected by a groove attachments along their anterior edges. A large number of pseudotracheae run from the inner margin of oral lobes to the outer borders. The number of pseudotracheae on each lobe is generally 12 (12+12=24)

The thorax is roughly triangular and has well developed mesonotum. The wing venation has been described in the terms proposed by Comstock and Needham (loc. cit) The hind pair of wing is modified into halteres which are considered to represent the rudimentary meta-thoracic wing Each of the three pairs of legs is composed of coxa, trochanter femur tibia, tarsus, claw and pulvillus.

The abdomen is shown to consist of eight segments in the male and nine in the female, in both, the first five segments are visible. The external genitalia of the male is formed of three abdominal segments. In female the genitalia is made up of four abdominal segments, being telescoped one within the other so that only the terminal tubercles are visible from the exterior

The alimentary canal is looped upon itself and its length exceeds that of the body It is divisible into three main regions, viz., Stomodaeum, Mesenteron and Proctodaeum. The stomodaeum consists of pharynx, oesophagus, crop and proventriculus There are two long, simple salivary glands. The upper half portion of mesenteron forms the beaded appearance and rest half is tubular and convoluted in form. Two malpighian tubules arise at the junction of mid and hind gut. Each tube shortly divides into two tubules. The proctodaeum consists of ileum, colon and rectum which opens into the anus.

The female reproductive system comprises of a pair of ovary a pair of oviducts, common ducts, vagina, a pair of accessory glands and a pair of spermathecae Each ovary consists of six ovarioles. The male organ consists of a pair of reddish testes, a pair of vasa-deferentia, ejaculatory duct, ejaculatory sac, a pair of accessory glands and aedeagus.

The central nervous system has only two well developed ganglia, viz supra oesophageal and thoracico-abdominal ganglia.

The host plants recorded from the various parts of the world have been listed. All the host plants belong to the family leguminoceae. *Phaseolus mungo* *Glycine hispida*, and *Phaseolus aconiti*[illegible] are the first record of the occurrence of this insect in India.

the anterior spiracles and the last segment carries the posterior spiracles and the anus Each abdominal segment of the body bears a transverse furrow which sub-divides it into two and therefore to all appearance one notes double the actual number of segments.

The head appears as bilobed when viewed from above. There are two oral hooks which lie in the lateral pouches of the oral cavity The maggot applies them for tunneling through the seeds for breaking down the cotyledon in order to liberate the juice which forms its food.

Just after the head, lies the thoracic region and abdomen which consists of three segments viz pro meso and metathorax. The segments are marked by circular swellings. The first thoracic segment has a pair of anterior spiracles situated near its posterior border The last body segment is larger and bears the posterior spiracles. The anus is situated on the rear part of the ventral surface between a pair of lobes.

The alimentary canal is divisible into fore gut, mid gut and hind gut. The fore gut consists of mouth pharynx, oesophagus and globular proventriculus. The common salivary duct opens into the pharynx. The mid gut or ventriculus is simple tube and forms most part of the alimentary canal. There are four bulbory gastric caecae at the anterior end of the mid gut The hind gut consists of ileum colon and rectum which opens at the anus. There are four malpighian tubules which arise in pairs on each side of the gut from a common tube

The nervous system consists of a brain which has two lobes and a compound ganglion. The nerve trunks arise from the compound ganglion to various parts of the body

The tracheal system consists of two dorsal trunks extending between the anterior and posterior pairs of spiracles a dorsal commissure in each segment connects the main trunks. The first and last commissures are more conspicuous than others. The viscera and body wall get trachae from lateral branches of dorsal trunks The lateral branches are connected by a series of longitudinal trunks except the first three

The head of adult *Agromyza obtusa* Mall is highly modified. It is strongly convex in front the posterior surface being almost flat and slightly concave. The genae consist of large compound eye which cover almost the whole of the antero-lateral regions of the head. The epicranium on the posterior surface is flat and on the anterior surface it is convex. It contains three ocelli situated on a slightly raised ocellar triangle The antennae arise from the lower end of the frons Antenna consists of three segments scape pedicel and flagellum. Flagellum bears the arista Arista tapers towards the apex and is hairy [illegible]

whole of the antenna. At the anterior region of the face there are two fronto-orbital bristles tapering towards mouth.

The proboscis consists of two parts, the proximal rostrum and a distal half of the proboscis proper which bears the oral lobes. The sides of the haustellum are formed by the overlapping labrum-epipharynx and labium hypopharynx. The two oral lobes are normally connected by a groove attachments along their anterior edges. A large number of pseudotracheae run from the inner margin of oral lobes to the outer borders The number of pseudotracheae on each lobe is generally 12 (12+12=24)

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The damage is caused chiefly by the maggots, but adult females also damage the leaves by puncturing them. The leaves that are punctured turn yellow, droop down and dry up and later the apical portion of the stem and through which the maggot has entered, meet the same fate. Gradually whole plant begins drooping and ultimately dies. The whole process takes 10 to 1[illegible] days by which time the maggot pupates and does not require any food.

The adults copulate 2 to 8 days after emergence Copulation usually occurs in the morning hours of the day The oviposition lasts for 4 to 11 days. The eggs are generally laid on the lower surface of the leaves some-times on the stem, petiole, and rarely on the upper surface of leaves. The fe-male fly makes a cavity roughly elliptical, in which it inserts an egg under the epidermis of the leaf. The fly then turns and sucks the exuding sap. Out of many punctures made by the female only very low percentage contains eggs and rest are probably used for feeding

The egg is elongated, white in colour The incubation period of the egg varies from 2 to 4 days depending on the season, shortest in March to April and longest in December There are three larval instars and the total larval period varies from 9 to 12 days the shortest (7 days) in April and the longest (12 days) in December and January

The pupa is barrel shaped. The freshly formed pupa is yellow and turns dark brown before the emergence of the fly Pupation takes place in the larval gallery but in the beginning of the attack (*i.e.*, seedling stage of pea) in the underground part of stem The pupal period varies from 5 to 9 days. The least in March April and highest in December and January Under field condi-tions the pupal period is prolonged upto 1 month or more.

The flies emerge by breaking the puparium at the anterior end through a circular opening and forcing its way out of the stem. A fully formed fly is metallic black in colour The wings are hyaline. The female is bigger than the male. The adults are very active and fly away on slight disturbance They are seen congregating under the leaf in the morning

The females live much longer than males, except when starved. It was observed in the laboratory that males live for 6 to 18 days after emergence and females for 8 to 22 days. In starved conditions both the sexes lived only for two days. Under laboratory the flies were fed on sugar cubes in rearing jars and chimneys.

The fly *Melanagromyza phaseoli* Coq is active for about 240 days in a year and one brood takes about 70 days. There are 8 to 9 generations in a year.

The flies are in abundance during two seasons of the year in the first in October November and the second in March-April, when food plants are abun-

able in large quantities. The maggots and pupae hibernate in cooler months, *i.e.* December January The adult flies undergo aestivation during May June. Female flies are found in large numbers than males

Inspite of the pea plants the fly also attacks other host plants from July October such as moth (*Phaseolus aconitifolius*) soya bean (*Glycine hispida*) and lobia (*Vigna catjang*)

Trials on chemical control of *Phytomyza atricornis* Meigen on peas were conducted for two years continuously while on *Agromyza obtusa* Mall. on arhar for one year Out of five insecticides tried in the form of emulsion, Endrin was found most effective in controlling the adult flies and maggots.

STUDIES IN THE FRUIT DROP OF MANGO
(*MANGIFERA INDICA* L.)

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Drop of the fruits at various stages of their development in most of the fruits has been a serious problem with the fruit-growers all over the world. This problem has been studied in detail particularly in deciduous fruits, viz apple (*Pyrus sylvestris* Mill.) pear (*Pyrus communis* L.) and peach (*Prunus persica* Batsch) and also in citrus specially in Sweet Oranges (*Citrus sinensis* Osbeck)

From the review given in the present work, it can be seen that no adequate studies on the causes and control of the fruit-drop in mango (*Mangifera indica* L.) have been done. It was, therefore with a view to study (1) the nature of fruit-drop, (2) its possible causes and (3) the control measures that two commercial varieties Dashehari and Langra were selected for the investigations mentioned above.

The studies reveal that in Dashehari the percentage of hermaphrodite flowers in the terminal, middle and the basal regions of the panicle is 45 06, 43 77 and 43.13% respectively It is however 62 89% in the terminal, 59.84% in the middle and 59 79% in the basal regions of the panicle in variety Langra. On an average the percentage of bisexual flowers in a panicle is 44.03 and 60.94% in Dashehari and Langra respectively The sex-ratio of the male and hermaphrodite flowers works out 1.37 1 in Dashehari and 0 64 1 in Langra. It has also been observed that the heaviest fruit-set is in the terminal region of the panicle which is followed by the middle and then by the basal region in both the varieties. Per panicle initial fruit-set (mustard stage) is 1.82% in Dashehari and 2.53% in Langra. This initial fruit-set is sufficient to carry a heavy crop But in these varieties the severe fruit-drop starts from mustard stage of the fruits and causes the shedding of 93.4 to 97 60 % in Dashehari and 98 10 to 99 18 % in Langra upto their maturity The observations on the whole tree of these varieties exhibit almost similar tendency of fruit shedding

While studying the extent of the fruit-drop under different size-grades, it has been found that shedding of small fruits upto 0.5 cm. size is the heaviest in Dashehari and the severity of fruit-fall is equally heavy of the fruits upto 1 0 cm. size in Langra. As the fruits gain in size, their shedding decreases considerably After stone-formation, the fruit-fall is almost negligible in these varieties.

It has also been observed that unlike the normal fruits, those likely to drop the following week, show inhibition in their growth. This is suggestive of the fact that the causes responsibl for the fruit-shedding set in ahead of the actual fruit-fall.

The detailed studies on the causes of fruit-drop in mango have been worked out. It is observed that drop due to shrivelling of fruits varies from 4.63 to 32.49% in Dashehari and 20.81 to 26.75% in Langra. Diseases and insect pests are responsible for the shedding of 0.35 to 2.24% in Dashehari and 2.61 to 19.02% in Langra. The high fruit-fall in variety Langra is, however *mainly attributed to* the incidence of anthracnose and *fruit-rot diseases*. Sun-burn and fruit-splitting are responsible for 0.35% shedding in Dashehari and 0.07 to 2.30% in Langra

The occular examination of the dropped fruits indicate that the ovular defects bring about drop of 48.72 % fruits in Dashehari and 39.08 % in Langra. Of these, the average total drop due to shrivelling and degeneration of ovary is 43.24 % and 30.12% in Dashehari and Langra respectively Abnormal development of cotyledons and of the embryo is responsible for an average total drop of 4.50% in the variety Dashehari No such abnormality was noticed in Langra. Suppression or complete absence of cotyledons, however cause fruit fall of 2.70 to 6.16% fruits in Langra only

Thus, it is apparent that ovular abnormalities are responsible for about half of the total fruit-drop

Detailed embryological studies were made to investigate the normal embryo development and ovular defects. These observations show that in mango the ovule is unitegmic, crassinucleate and basal. It finally occupies a lateral position due to one-sided growth of the ovary The embryo sac development is of polygonum type. The mature embryo-sac is 8-nucleate. Zygote rests upto the mustard stage of the fruits and after its first division, the subsequent development is quite rapid Finally the pro-embryo develops into a dicotyledonous embryo which consumes the endosperm during the period of its development. In these varieties, the primary endosperm nucleus divides rapidly by free nuclear division and upto the resting stage of zygote many endosperm nuclei are formed.

It has been observed that the degeneration starts as early as the 4-nucleate stage of the embryo sac and continues upto the formation of the complete egg apparatus in the flower Such degenerations also occur in fruits and affect the embryo and the ovary-wall.

The anatomical studies were also conducted on the pedicel and mode of abscission layer formation These investigations show that the anatomy of the pedicel is similar to that of the stem The epidermal cells are covered by thick cuticel and they are radially elongated The cortical and pith cells are parenchymatous and th resin canals form lysigenously

The pedicels of flowers and fruits have an abscission zone which is composed of small elliptical cells arranged in irregular rows. These cells are meristematic and rich in cytoplasm.

The separation layer always forms in an irregular way in the abscission zone at all the stages of fruit-development. The cells detach through their middle lamellae and the xylem tissues are torne by the weight of the fruit.

With a view to determine calcium oxalate, calcium pectate and other compounds, micro-chemical studies of the pedicle and abscission zone were also undertaken from early stages to the maturity of the fruits. These investigations indicate that the cells of the pedicels and abscission zone of the unripe fruits have pectic compounds, pectic acid and calcium while those of the ripe fruits have pectic acid and calcium oxalate only. Therefore, it is evident that the micro-chemical compounds are transformed into calcium oxalate in the abscission zone of the ripe fruits. These changes cause the easy detachment of the ripe fruits.

Out of the control measures tried for checking the fruit-drop sprays of 15 ppm. of NAA and 10 ppm. of 2,4-D reduce the fruit-fall from 18.00 to 20.19% in Dashehari. In variety Langra, spray of 2,4-D in concentration of 10 ppm. proved effective in checking the fruit-shedding from 21 98 to 25 15% in comparison to control. These plant-regulators also improved the quality of the fruits.

The foliar sprays of calcium and magnesium salts and soil application of gypsum though effective in checking the fruit-drop to an extent of 10.50 % are not so promising as the NAA and 2 4-D

STUDIES ON THE EFFECT OF THREE LEVELS OF NITROGEN ON THE GROWTH AND CROPPING OF MANDARINS (*CITRUS RETICULATA BLANCO*) AND SWEET ORANGES (*C SINENSIS OSBECK*) BUDDED ON DIFFERENT ROOTSTOCKS*

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1 A complete review dealing with the effects of nitrogenous fertilizers and manures applied to the soil of the citrus orchards on vigour and cropping behaviour of citrus trees and their subsequent effect on the soils has been prepared. It has been argued that the conduct of such trials is highly essential under Indian conditions for the benefit of the orchardists, because of the absence of data in this context.

2. An experiment dealing with the influence of three levels of nitrogenous fertilizer on vigour cropping behaviour and leaf composition of two varieties each of Sweet Oranges (*Citrus sinensis* Osbeck) and Mandarins (*C reticulata* Blanco) budded on two different rootstocks namely Florida Rough and Karna Khatta, has been described. The varieties selected for this study were Mosambi and Navelencia among Sweet Oranges and Srinagar and Rangtra among Mandarins. The levels of the fertilizer were N_1 (2 lb. of nitrogen per tree of 10 years of age) N_2 (3 lb of nitrogen per tree of 10 years of age) and N_3 (4 lb of nitrogen per tree of 10 years of age) The exact dose determined on the basis of the age was increased as the trees advanced in age.

Half of the nitrogen was applied in form of Castor Cake in the month of October and the remaining half was provided from ammonium sulphate (20 6% N) The latter was applied in two equal instalments in the months of January and June.

The effects of the above doses of the fertilizer on macro-nutrient status of the orchard soil were also determined and described.

3 Different doses of the fertilizer did not differ significantly from each other in increasing the vigour of the trees in any of the years. This was due to the fact that the tree had reached the bearing stage and the fertilizers applied were diverted towards the formation and development of flowers and fruits.

4 In Sweet Oranges different doses of the fertilizer produced significant response in respect of total number of flowers, percentage of initial fruit set, and the yield of the ripe fruits (expressed in weight as well as in number of the fruits) N_3 treatment produced the highest number of flowers followed by N and then by N Similar trends were observed for percentage initial fruit set too

* This is an abstract of the thesis submitted and approved for the Ph. D degree of the Agra University in the year 1960.

during 1957 58 and 1958-59 but in 1959-60 N_2 showed the best response followed by N_3 and then by N_1

N_2 gave the best result for the weight of the fruit but N_3 was superior to N_2 in the number of fruits during the first two years of the trial. During the last year of the observation, N_2 superseded N_3 in number of fruits and N_1 had the lowest value in all the cases

As regards Mandarins different doses of the fertilizer produced significantly different response in respect of all the factors described above for Sweet oranges. Here also N_2 was the supermormost and N_1 the last of all.

The data concerning the effect of fertilizers on fruit growth, fruit drop and the time of ripening of fruits were not statistically analysed Out of these factors, clear-cut differences due to fertilizers were observed for fruit growth only where N_2 had the best effect in respect of both the scions (Sweet Oranges and Mandarins)

The fertilizers did not have marked effect on time of ripening of fruits as judged by their T S S /Acid ratio

5 Although the data concerning the effect of the fertilizers on the quality of the fruits were also not statistically analysed, some broad conclusions have been drawn on the basis of the information collected in this context.

In Sweet Oranges, N_1 produced the heaviest weight per fruit and the highest percentage of juice followed by N_3 and then by N_2. N_2 showed the highest percentage of rind and rag and lowest storage quality of fruits. N_1 fruits had the best storage quality and N_3 ones were next to them.

Fertilizers affected the quality of the Mandarin fruits also. Here N_2 produced the lowest juice percentage and storage quality of the fruits and the rind and rag percentage was the highest in this case N_1 showed the best weight per fruit highest juice percentage and best storage quality of fruits. N_3 fruits held intermediate position in respect of the storage quality

6 Nitrogenous fertilizers did not show marked effect on the N content of leaves nor did they affect the total nitrogen and available P_2O_5 and K_2O contents of the soil. The former effect was explained to be due to the age of the leaves at which samples were drawn for analytical purposes.

7 Interactions between fertilizers, the stocks and the scions were also found significant to some extent in certain years. However such interactions were not significant for the yield (except in case of Sweet Oranges for the weight of the fruits) It appears that the nitrogen requirements for citrus trees varied from scion to scion and from stock to stock except in regard to the yield of the ripe fruits.

8 It has been concluded that while N_1 dose proved inadequate, N_3 was excessive or toxic N_2 treatment proved to be the best in these studies. These results were true for both the varieties of citrus (Mandarins and Sweet Oranges)

9 In Mandarins, differences between the scions were also found significant for the vigour of the trees, total number of flowers average number of fruits and the yield of the ripe fruits. Generally Srinagar proved superior to Rangtra in all these respects.

In Sweet Oranges the differences between the scions were significant for all the factors described above under Mandarins except the vigour of the trees. Here, Mosambi was usually found superior to Navelencia.

10. With Mandarins the differences between the stocks were also found significant for the vigour of the trees. Florida Rough had better performance than Karna Khatta in this respect. But the latter rootstock had significantly higher number of flowers yield and storage quality of fruits and higher pectin content of rag and peel of ripe fruits

In Sweet Oranges, the differences between the stocks were significant in respect of total number of flowers, average number of fruits set and the yield of the ripe fruits. In this case too Karna Khatta was generally superior to Florida Rough.

From these observations it was concluded that Karna Khatta was universally superior to Florida Rough from commercial point of view

11 Interactions between stocks and scions were also found significant in certain cases. This shows that the suitability of stocks differed from scion to scion.

STUDIES INTO THE EFFECTS OF SOURCE, PLANT REGULATOR TREATMENT AND PLANTING ENVIRONMENT ON CITRUS CUTTINGS

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The propagation of fruit plants by cuttings is gaining much more importance throughout the World and a great advancement has been made in different countries in this direction in recent years. In India, however very little work has been done so far on this aspect The factors governing the success of cuttings may be grouped under three heads *i.e.*, source treatment between collecting and planting and the planting environment. The various species of citrus not only show great variations in vigour but the water sprouts which are so common in them are much more vigorous than the normal shoots Thus, citrus species afford great possibilities for understanding the underlying principles and practical application of different groups of factors.

In view of the above a series of six experiments were conducted to study the effects of source, plant regulator treatment and planting environment on the stem cuttings of Karna Khatta (*Citrus karna* Raf.) Sweet lime (*Citrus limettioides* Tanaka) and Kagzi lime (*Citrus aurantifolia* Swingle) at Balwant Rajput College, Bichpuri, Agra during 1957 59

SITE AND SOURCE OF CUTTINGS

The experiments were conducted under field conditions as well as in pots. The field experiments were conducted on a uniform piece of land in the nursery area of the garden, and pot experiments in the propagation house near the college laboratories. The cuttings were obtained from young nursery plants of Karna Khatta and bearing trees of this as well as sweet and kagzi lime trees of the orchard. Some trees of the latter two were dehorned in February 1957 for providing cuttings of this source

PREPARATION AND PLANTING OF CUTTINGS

The hardwood cuttings of about 9 length were prepared by giving straight and slanting cuts at basal and distal ends respectively and removing all the leaves. Softwood tip cuttings, on the other hand, of about 4 length were prepared by giving a straight basal cut and removing about two pairs of basal leaves. The treatment of the cuttings with plant regulator (indolebutyric acid) consisted in momentarily dipping the basal ends of the cuttings to about 1 length in their respective solutions prepared in 50 per cent alcohol and allowing

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the alcohol to dry before planting The cuttings were then planted with the help of swords of appropriate size (6" deep holes for hardwood and 1" for softwood cuttings) for them at the demarcated points in the beds or in pots. Watering was done immediately after planting

Care and Supervision

All possible cares about irrigation, weeding and hoeing, and protection against insect pests and diseases were taken throughout the experimental periods in field as well as pot experiments.

Technique of Study and Presentation of Data

The observations of the above ground parts of the cuttings regarding survival and sprouting were recorded at fortnightly intervals. The cuttings for the final study were carefully lifted, washed and then studied individually for various root and shoot characters. After study the hardwood cuttings were planted in beds whereas softwood ones in bigger pots and slowly hardened. Anatomical studies of root initiation were also done in various types of cuttings.

The data collected at final recording were analysed statistically by the method of analysis of variance where possible The trends of survival and sprouting etc. were studied with the help of curves The following are the details of individual experiments conducted and the results achieved during the course of investigation

Experiment I

Studies into the Effects of Source and Rooting Medium on Karna Khatta Hardwood Cuttings

The influence of seven rooting media (sand leaf mould and soil, and their mixtures) on the performance of cuttings of Karna Khatta obtained from young nursery plants and bearing trees, was studied during rains of 19[illegible] and 1958 The cuttings were planted in 7"×4" pots after giving a basal concentrated dip of 1000 p p m IBA. The design of the layout was randomized block with four replications. There were 15 cuttings, five for intermediate studies on root initiation and ten for final study in each plot.

Cuttings obtained from young nursery plants gave much better results than those of bearing trees in all respects because of a better speed of reaction as observed by the amount of callus and root initiation in the former in comparison to the latter The percentage of rooting in the former was 60 3 and 73 6 as against only 28 6 and 20 0 in the latter during 1957 and 1958 respectively

Of all the media a 50 : 50 mixture of sand and leaf mould proved quite satisfactory for nursery cuttings because of better rooting capacity of sand accompanied with better survival and establishment capacities of leaf mould. For the cuttings from bearing trees sand alone proved better since in this case rooting started very late and, thus there was no problem of death after rooting till the final recording

During 1957 when rainfall was normal and there was not much problem of drainage, the media containing soil as a constituent did not prove much inferior than others, but during 1958, when rainfall was very heavy the results in such media were very poor. Further the superiority of rainy season cuttings over those of bearing trees during 1958 was more marked. The probable reason for this was a sudden change in weather during this year after a few weeks from planting when the cuttings of nursery plants, due to presence of well developed root systems, could stand this change and those of bearing trees died at a very fast rate.

Experiment II

Studies into the Effects of Maturity of Wood and Concentrations of Indolebutyric Acid on Karna Khatta Cuttings

The influence of varying concentrations of IBA (0, 500, 1000, 2000 and 4000 p.p.m.) applied by the quick dip method, on hard and semi-hardwood cuttings of Karna Khatta (young nursery plants) was studied during 1957 and 1958. The cuttings were planted in beds laid out by the randomized block design. Fourteen cuttings planted in two rows, ten experimental and four border formed a plot. Two border rows on the remaining two sides of each block were also planted.

The cuttings in 1957 were planted in the month of September and were finally studied after 26 weeks from planting, whereas during 1958 the cuttings were planted in July and were finally studied only after 16 weeks from planting, since the growth was much better and faster in this year.

July planting proved far superior to September one in all respects (67.6 and 16.6 per cent rooting respectively) and only a slight improvement in the rooting of semi-hardwood cuttings by an application of 500 p.p.m. was noted during both the years, higher concentrations proving deleterious, particularly in 1957. The interesting point, however, was the increase in number of roots on the cuttings with every increase in the concentration of IBA. It seemed that the cuttings, which could stand higher concentrations, took the advantage for earlier and better rooting.

Experiment III

Studies into the Effects of Source and Plant Regulator Treatment on the Performance of Sweet and Kagzi Lime Hardwood Cuttings

The performance of hardwood cuttings of sweet and kagzi limes obtained from three types of shoots *i.e.* shoots from dehorned trees, water sprouts from normal trees and normal shoots from normal trees, treated with varying concentrations of IBA (0, 500, 1000, 2000 and 4000 p.p.m.) by the quick dip method

in 50 per cent alcohol, was studied during 1957 and 1958 The cuttings of the first year were planted on 29th August and studied on 20th to 22nd March, 1958 after 29 weeks from planting whereas those of second year were planted on 18th and 19th February and were finally studied on 16th July 1958 after 20 weeks from planting In this experiment one row of 12 cuttings formed a plot and other things were similar to the layout of previous experiment.

The performance of the cuttings planted in August, 1957 was far superior to those planted in February 1958 due to the moist and moderate weather [illegible] able for the cuttings planted in former season Sweet lime proved better than kagzi lime as a source of cuttings probably because of inherent variations. Dehorning of the trees about 6 months before taking of the cuttings induced a similar invigorating effect in the new shoots as was found in the water sprouts and both the types of shoots proved much better source of cuttings than the normal ones of sweet lime. Indolebutyric acid at 2000 p.p.m. improved the rooting of cuttings of all the three types and resulted in as high as 83 per cent rooting in the first two sources.

Experiments IV and V

The performance of kagzi lime cuttings in the third experiment was very poor from the very beginning and a very high percentage of them died within two months from planting Two more experiments were, therefore conducted to increase the success of kagzi lime cuttings. In the fourth experiment (*Periodicity in the performance of kagzi lime hardwood cuttings*) the cuttings were planted in all the twelve months *i.e.*, from January to December 1958 after giving a basal concentrated dip of 1000 p p m. IBA. The cuttings were obtained from all the sources of the third eperiment The results however in all the months were very poor and only slight rooting was noted in the cuttings planted during August, September and October (4 2, 10 0 and 1 0 per cent respectively) There was no much difference in the rooting of the cuttings from the three sources, since the rooting on the whole was very poor

In the fifth experiment (*Studies into the effects of planting date [illegible] age of the shoots on the performance of kagzi lime hardwood cuttings*) ringing treatment was tried as an aid in rooting of cuttings. An experiment was conducted to study the performance of one and two year old shoots, with and without [illegible] when planted as hardwood cuttings in the last week of every month from [illegible] to December 1958 Ringing was done two weeks before planting in each case and a basal dip of 500 p p m. IBA in 50 per cent alcohol was also given to the cuttings. The cuttings were finally recorded on April 11 1959

Ringing of the source shoots increased the rooting percentage [illegible] could not improve their establishment Of the six planting months [illegible] occurred only on the cuttings planted in July August and October (16 [illegible] and 1 3 per cent respectively [illegible] and [illegible] year old shoots [illegible] though similar [illegible]

mg, proved better than one year old shoots in respect of establishment. The effect of ringing treatment was marked in July and August but negligible later on. Though rooting in some of the treatments reached upto 45 per cent, the establishment was very poor (upto 12 5 ,) and, thus, this experiment was also not very successful.

Experiment VI

Studies into the Effects of Planting Time and Concentrations of Indolebutyric Acid on the Performance of Softwood Cuttings of Kagzi Lime

The failure of the hardwood cuttings in 3rd, 4th and 5th experiments led to some attempts of its propagation by softwood cuttings, which had given some promising results in this species in an another experiment. This investigation was, therefore, conducted to study the influence of planting dates at fortnightly intervals, during spring as well as rains, on softwood cuttings of kagzi lime During spring the planting commenced from January 26, 1958 and the cuttings were obtained from November flush. The cuttings were finally examined in the first week of July 1958 after 23 weeks from first planting In the rains, on the other hand, the cuttings were taken f om June flush and planting began from July 12, 1958 The final study was done after 14 weeks from first planting in the third week of October The cuttings were treated w th 0 250, 500 and 1000 p.p.m. IBA. in 50 per cent alcohol by the quick d p method.

The cuttings were planted in 3" earthen pots filled with 50 50 sand and sieved leaf mould. There were four replications and 10 cuttings were put under each plot. The pots were put in polythene (500 gauge) covers in randomized block design under the shade of lath house

A humid and moderate rainy season proved far superior to the spring and summer months in all respects. During rains the cuttings planted towards the end of July or in August performed much better (94 to 98 % rooting and 84 to 93% establishment) than those planted in early July (74 % rooting and 51% establishment)

There was no effect of the treatment of cuttings with indolebutyric acid at any concentrations tried here and the cuttings receiving no plant regulator treatment were as good as those receiving such treatment.

Special Studies

Anatomical studies of root in tiation showed that the roots originated from any tissue beginning from the pericycl upto the cambium by the activity of parenchymatus or meristematic cells. There was not much difference in the hard and semi-hardwood cuttings except for the ease in rooting in the latter case. It appeared that the roots imerged through the cortex mechanically Nodes produced roots more frequently than internodes because of better nutritive conditions and greater amount of parenchyma.

Callusing and rooting though quite distinct processes, had a great effect upon each other The cuttings producing callus profusely also rooted very readily and those producing nil or less amount of callus rooted very rarely in most of the cases.

Conclusions

The experiments, conducted to study the influence of source, pl... regulator treatment and planting environment on stem cuttings of three species of citrus i.e Karna Khatta, sweet lime and kagzi lime have yielded some very interesting and practical results Hardwood cuttings of Karna Khatta, obtained from young juvenile plants and planted during early rains in pots filled with 50 50 mixture of sand and leaf mould, gave on average 76 per cent rooting and 60 per cent establishment, and in beds 67 8 per cent rooting and 65 3 per cent establishment. As regards plant regulator treatment only semi-hardwood cuttings benefitted from a basal concentrated dip of 500 p p m. IBA, whereas hardwood cuttings did not need any treatment.

Hardwood cuttings of sweet lime obtained from vigorous shoots like water sprouts (from September-October flush) and those from dehorned trees and planted during rains after treatment with 2000 p.p.m IBA, by the concentrated dip method, gave highly satisfactory rooting of 85 per cent and practically all of them established into new plants.

Hardwood cuttings of kagzi lime proved very shy to root but softwood cuttings showed very promising results. The latter type of cuttings obtained from 6 week to 10 weeks old shoots of June flush planted in 5" earthen pots under polythene covers in partial shade of lath house towards the end of July or in August gave from 94 to 98 per cent rooting and 81 to 93 per cent establishment.

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SOME PROBLEMS IN OPERATIONAL CALCULUS OF TWO OR MORE VARIABLES AND PROPERTIES OF FOURIER BESSEL'S TRANSFORM ANALOGOUS TO HANKEL'S TRANSFORM*

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Chapter 1

Certain Rules of Generalized Laplace Transform

1 Generalization of the wellknown laplace Transform

$$\phi(p) = p\int_0^{\infty} e^{-pt} f(t)\, dt \qquad 1$$

has been given from time to time and we have introduced the transform

$$\phi(s) = s\int_0^{\infty} e^{-\frac{st}{2}} (st)^{m-\frac{1}{2}}\ M_{k,m}\ (st)\, f(t)\ dt$$

which is symbolically written as

$$f(t) \xrightarrow[m]{k} \phi\ (s)$$

In this connection the following properties are also given which are the kernels involved in this transform.

(i) $\frac{d}{dx}\left[x^{m-\frac{1}{2}}\, e^{-\frac{x}{2}} M_{k,m}\ (x) \right] = 2m\, x^{m-1}\, e^{-\frac{x}{2}}\ M_{k+\frac{1}{2},\, m-\frac{1}{2}}(x)$

(ii) $\frac{d^n}{dx^n}\left[x^{m-\frac{1}{2}}\, e^{-\frac{x}{2}} M_{k,m}\ (x) \right] = \frac{\lfloor 2m}{\lfloor n} x^{m-\frac{1}{2}(n+1)} e^{-\frac{x}{2}} M_{k+\frac{1}{2}n,\, m-\frac{1}{2}n}(x)$

(iii) $\int_0^{\infty} x^{m-\frac{1}{2}}\, e^{-\frac{x}{2}}\ M_{k,m}\ (x)\, dx = \Gamma(2m+1) \sum_{r=0}^{\infty} \frac{(m-k+\frac{1}{2})_r}{\lfloor r}$

(iv) $\int_0^{x} x^{m-\frac{1}{2}}\, e^{-\frac{x}{2}}\ M_{k,m}\ (x)\ dx = \frac{1}{2m+1} x^{m}\, e^{-\frac{x}{2}}\ M_{k-\frac{1}{2},\, m+\frac{1}{2}}(x)$

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*This is an abstract of the thesis submitted and approved for the Ph. D degree of the Agra University in the year 1960

†My thesis contains the treatment of the operational calculus in two or more variables, making use of Laplace Transform and its generalisations in two or more variables and some properties of Hankel Transform. This forms the main part of the thesis. It also contains some results involving legendre polynomials which may be called the subsidiary part.

—Author

(v) (a) $\frac{d}{s\,dt}\left[(st)^{m-\frac{1}{2}} e^{\frac{st}{2}} M_{k,m}(st)\right] = 2m(st)^{m-1} e^{\frac{st}{2}} M_{k+\frac{1}{2},\, m-\frac{1}{2}}(st)$

(b) $\frac{d}{t\,ds}\left[(st)^{m-\frac{1}{2}} e^{\frac{st}{2}} M_{k,m}(st)\right] = 2m(st)^{m-1} e^{\frac{st}{2}} M_{k+\frac{1}{2},\, m-\frac{1}{2}}(st)$

(vi) $s\int (st)^{m-\frac{1}{2}} e^{\frac{st}{2}} M_{k,m}(st)\,dt = \frac{1}{2m+1}(st)^{m} e^{\frac{st}{2}} M_{k-\frac{1}{2},\, m+\frac{1}{2}}(st)$

2. The following rules of the generalized Transform have been obtained, which are valid under the conditions stated therein. Here we write $\phi(s)$ in the form $\phi(s,k,m)$ and then

$$\phi(s) = \phi(s,k,m) = s\int^{\infty} (st)^{m-\frac{1}{2}} e^{\frac{st}{2}} M_{k,m}(st) f(t)\,dt$$

Which is written symbolically as

$\phi(s;k,m) = M[f(t);k,m]$

Now if $\phi(s;k,m) = M[f(t);k,m]$ then

(i) $M\left[f(\beta t);k,m\right] = \phi\left(\frac{s}{\beta};k,m\right)$

(ii) $M\left[t\frac{d}{dt}f(t);k,m\right] = -s\frac{d}{ds}\phi(s;k,m)$

(iii) $M\left[\left(t\frac{d}{dt}\right)^{n} f(t);k,m\right] = \left(-s\frac{d}{ds}\right)^{n}\phi(s,k,m)$

(iv) $M\left[\int_0^t \frac{f(x)}{x}\,dx;k,m\right] = \int \phi(x;k,m)\frac{dx}{x}$

(v) $M\left[\int_0^t \frac{f(x)}{x}\,dx;k,m\right] = \int^{\infty}\phi(x,k,m)\frac{dx}{x}$

(vi) $M\left[\frac{d}{dt}f(t);k,m\right] = -2ms\,\phi(s;k+\frac{1}{2},m-\frac{1}{2})$

Provided $\left[(t)^{m-\frac{1}{2}} e^{\frac{st}{2}} M_{k,m}(st)\,f(t)\right]_0^{\infty}$ is zero

(vii) $M\left[\int_0^t f(x)\,dx;k,m\right] = -\frac{1}{2(m+1)s}\phi(s;k-\frac{1}{2},m+\frac{1}{2})$

Provided $\left[(st)^{m} e^{\frac{st}{2}} M_{k-\frac{1}{2},\,m+\frac{1}{2}}(st)\int_0^t f(x)\,dx\right]^{\infty}$ is zero.

(viii) $M\left[tf(t);k,m\right] = -\frac{s}{2m+1}\frac{d}{ds}\left[\frac{1}{s}\phi(s;k-\frac{1}{2},m+\frac{1}{2})\right]$

$$(ix)\quad M\left[\frac{f(t)}{t}\quad k,m\right]=-(2ms)\int_{s}^{\infty}\phi(x\quad k+\tfrac{1}{2},m-\tfrac{1}{2})\frac{dx}{x}$$

$$(x)\quad M\left[\frac{d^{n}}{dt^{n}}\quad f(t)\quad k,m\right]=(-1)^{n}\,s^{n}\frac{\lfloor 2m}{\lfloor 2m-n}\,(\phi s\quad k+\tfrac{1}{2}n,m-\tfrac{1}{2}\,)$$

$$(xi)\quad M\left[\int_{0}^{t}\int_{0}^{x}\int^{x}f(x)\quad(dx)^{n}\quad k,m\right]=\frac{\lfloor 2m+2}{\lfloor 2m+2+n}\,(-1)^{n}$$

$$\left(\frac{1}{s}\right)^{n}\phi\,(s\quad k-\tfrac{1}{2}\quad m+\tfrac{1}{2}n)$$

Provided $\left[(st)\ m-\tfrac{1}{2}\ e\frac{st}{2}M_{k,m}\,(st)\ F_{r}\,(x)\right]_{0}^{\infty}$ are all zero where

$$F_{r}\,(x)=\int_{0}^{t}\int_{0}^{x}\quad\int_{0}^{x}f(x)\ (dx)^{r}\quad r=1,2,3\quad\ldots n$$

$$(xii)\quad M\left[t^{n}f(t)\quad k,m\right]=\frac{\lfloor 2m+1}{\lfloor 2m+1+n}\,s\ \frac{d^{n}}{ds}\left[\frac{1}{s}\,\phi(s\quad k-\tfrac{1}{2}n,m+\tfrac{1}{2}n)\right]$$

$$(xiii)\quad M\left[\frac{f(t)}{t^{n}}\quad k,m\right]=\frac{\lfloor 2m}{\lfloor 2m-n}(-1)\quad s^{n}\int_{s}^{\infty}\int_{x}^{\infty}\ldots\int_{x}^{\infty}$$

$$\int_{0}^{\infty}\frac{1}{x}\quad\phi\,(x,k+\tfrac{1}{2}n\quad m-\tfrac{1}{2}n)\ (dx)$$

$$(xiv)\quad M\left[\int^{t}t\int_{0}^{t}\int_{0}^{t}tf(t)\,(dt)\quad k,m\right]=\left[\frac{\lfloor m+1}{\lfloor m+1+n}\right]^{n}$$

$$\frac{s}{2^{n}}\quad\left(\frac{1}{s}\ \frac{d}{ds}\right)\ \left[\frac{1}{s}\phi\,(s\quad k-n,m+n)\right]$$

where $\left[(st)\ m\ e\frac{st}{2}\quad M_{k}\ {}^{(st)}_{\frac{1}{2},m+\frac{1}{2}}\ F\ \ (t)\right]_{0}^{\infty}$ are all zero where

$$F_{r}(t)=\int_{0}^{t}t\int^{t}\int_{0}^{t}tf(t)\ (dt)^{r}\quad=1,2\ 3\quad\ldots n.$$

$$(xv)\quad M\left[\left(\frac{1}{t}\ \frac{d}{dt}\right)^{n}f(t)\quad k,m\right]=\frac{\lfloor 2m}{\lfloor 2m-}\quad s\int^{\infty}\int^{\infty}s\int_{s}^{\infty}$$

$$\phi\,(s\quad k+n\quad m-n)\ (ds)$$

Provided $\left[(st)\ m-\tfrac{1}{2}\ e\frac{st}{2}\quad M_{k,m}\,(st)\left(t\frac{d}{dt}\right)^{r}f(t)\right]^{\infty}$ are all zero,

$r=1,2,3\quad\ldots n$

3 (A) If $M\left[t^{\lambda} f(t) \quad k,m\right] = \phi(s \quad k,m)$ then

(i) $\lambda M\left[t^{\lambda} f(t) \quad k\,m\right] + M\left[t^{\lambda+1} \frac{d}{dt} f(t) \quad k,m\right]$

$$= -s \frac{d}{ds} \phi(s \quad k,m,\lambda)$$

(ii) $(\lambda+1) M\left[t^{\lambda} f(t) \quad k,m\right] + M\left[t^{\lambda+1} \frac{d}{dt} f(t) \quad k,m\right]$

$$= -2\,ms\phi\left(s \quad k+\tfrac{1}{2}, m-\tfrac{1}{2} \; \lambda+1\right)$$

Provided $\left[(st)^{m-\frac{1}{2}} \, e^{\frac{st}{2}} \, M_{k,m}(st) \, t^{\lambda+1} f(t)\right]_{t=0}$ is zero

(iii) $1/s\,\phi(s,k\,m\,\lambda) + 2ms\phi\left(s \quad k+\tfrac{1}{2} \quad m-\tfrac{1}{2} \quad \lambda+1\right) = -\frac{d}{ds}\phi(s\,k,m,\lambda)$

(B) If $M\left[t^{\nu} J_{\nu}(t) f(t) \quad k,m\right] = \phi(s \quad k,m,\nu)$ then

$$2ms\int_{s}^{\infty} \phi(s \quad k+1 \quad m-1,\nu)\, ds = \phi(s \quad k+1, m-1, \nu-1) + \phi$$

$$(s \quad k\,m,\nu-1) + 2(m+1)^2 s \int_{s}^{\infty} \phi(s,k+1,m-1,\nu)\, ds$$

4 To illustrate the applications of the above rules, I have solved 7 examples and thereby obtaining new transforms The extension of these rules have also been made to the generalized transforms of two variables

Chapter 2

On some integral Transforms in two variables

1 In section I of this chapter we have introduced the Generalised Laplace Transform

$$\phi(p,q) = pq \int_{0}^{\infty}\int_{0}^{\infty} e^{-\frac{xp}{2}-\frac{yq}{2}} (xp)^{-k_1-\frac{1}{2}} (yq)^{-k_2-\frac{1}{2}} M_{k_1+\frac{1}{2}} \quad (xp)$$

$$M_{k_2+\frac{1}{2}\, m_2} (yq) \, f(x,y)\, dx\, dy$$

in two variables analogue to Meijer's Transform This is symbolically denoted as

$$f(x\,y) \, \frac{k_1+\frac{1}{2}}{m_1} \quad \frac{k_2+\frac{1}{2}}{m_2} \quad \phi(p\,q)$$

This transform reduces to (i) $\phi(p,q) = pq \int_0^\infty \int_0^\infty e^{-xp-yq} f(x, y)\, dx\, dy$

where $k_1 = m_1$, $k_2 = m_2$

(ii) $\phi(p, q) = \dfrac{\lfloor n_1 \;\lfloor n_2 \;\Gamma(n_1)\;\Gamma(n_2)}{\Gamma(2m_1+n_1+1)\;\Gamma(2m_2+n_2+1)}\; pq \int_0^\infty \int_0^\infty$

$e^{-xp-yq}\, (xp)^{-n_1} (yq)^{-n_2} \times$

$L_{n_1}^{2m_1}(xp)\, L_{n_2}^{2m_2}(yq)\, f(x, y)\, dx\, dy$ where $k_1 = n_1 + m_1$, $k_2 = m_2 + n_2$.

(iii) $\phi(p,q) = 2^{2m_1+2m_2}\; \Gamma(m_1+1)\;\; \Gamma(m_2+1)\, pq \int_0^\infty \int_0^\infty e^{-\frac{xp}{2} - \frac{yq}{2}} (xypq)$

$I_{m_1}\left(\dfrac{xp}{2}\right) I_{m_2}\left(\dfrac{yq}{2}\right) f(x, y)\, dx dy$

When $k_1 = k_2 = -\frac{1}{2}$

(iv) $\phi(p,q) = \dfrac{(-1)^{n_1+n_2}\;\; \pi}{2^{n_1+n_2+1}\,\Gamma(3/2+n_1)\;\Gamma(3/2+n_2)}\; pq \int_0^\infty \int_0^\infty e^{-xp-yq}$

$(xp)^{-n_1} (yq)^{-n_2} \times$

$He_{2n_1+1}(2xp)^{\frac{1}{2}} \times He_{2n_2+1}\;(2yq)^{\frac{1}{2}} f(x, y)\, dx dy$

when $k_1 = n_1 - \frac{1}{4}$, $k_2 = n_2 - \frac{1}{4}$, $m_1 = m_2 = \frac{1}{4}$

(v) $\phi(pq) = \dfrac{(-1)^{n_1+n_2}\;\; \pi}{2^{n_1+n_2+1}\,\Gamma(\frac{1}{2}+n_1)\;\Gamma(\frac{1}{2}+n_2)}\; pq \int_0^\infty \int_0^\infty e^{-xp-yq} (xp)^{-n_1}$

$(yq)^{-n_2}\;\; He_{2n_1}\;[(2xp)^{\frac{1}{2}}] \times He_{2n_2}\;[(2yq)^{\frac{1}{2}}] f(x, y)\, dx\, dy$

2. In section II we have obtained the images $\phi(p\ q)$ of the following originals —

(i) $x^m y^n$ (ii) $\dfrac{\cos 2\sqrt{xy}}{\sqrt{xy}}$ $x<1$ $y<1$ (iii) $x^m y$ Sin $(x^{-\alpha} y^{-\beta})$

$x>1$ $y>1$ (iv) $x^m y\; J_\lambda^\mu (\frac{1}{4}\; x^{-\alpha}\; y^{-\beta})$ (v) $J\;(y\sqrt{x})\;\; I\;\;(y\sqrt{x})$

(vi) $x^{\frac{2m-n}{3}}\, y^{\frac{2n+m}{3}}\; J_{m,n}(3^2 \sqrt[3]{xy})$ (vii) $(xy)^\lambda\, e^{\frac{a}{xy}}$

(viii) $x^\mu\, y^\lambda\; J_\lambda\;(a\sqrt{x})\;\; J_\mu\;(b\sqrt{y})$

(ix) $x^{\mu_1} y^{\mu_2} J_{\nu_1}(ax) J_{\nu_2}(by)$

3 In section III we have obtained some new results deduced from the examples solved in Section II based on the following result —

(i) $f_1(x\ y) \frac{k_1+\frac{1}{2}}{m_1} \quad \frac{k_2+\frac{1}{2}}{m_2} \rightarrow \phi_1(p\ q)$

(ii) $f_2(x\ y) \frac{k_1+\frac{1}{2}}{m_1} \quad \frac{k_2+\frac{1}{2}}{m_2} \rightarrow \phi_2(p,q)$ then

$$\int_0^\infty \int_0^\infty \phi_1(x,y)\, f_2(x\ y) \frac{dx}{x} \quad \frac{dy}{y} = \int_0^\infty \int_0^\infty \phi_2(x\ y)$$

$$f_1(x,y) \frac{dx}{x} \quad \frac{dy}{y}$$

4 In section IV we have derived two important results —

(i) $\sum_{n=0}^{\infty} C_\eta^\lambda F_1\left[-n, -n-\nu \quad -n+1 \quad x\ y\right] \frac{k_1+\frac{1}{2}}{m_1} \quad \frac{k_2+\frac{1}{2}}{m_2} \rightarrow$

$$\sum^{\infty} C_\eta^\lambda \sum \frac{(-n)s+t(-n)\, s\, (-\nu)t\, \Gamma(m_1-k_1+s+1)\ \Gamma(m_2-k_2+t+1)}{(\lambda-n+1)_{s+t} \lfloor s \lfloor t \quad p^s q^t},$$

$${}_2F_1\left[\begin{matrix} m_1-k_1\ m_1-k_1+s+1 \\ 2m_1+1 \end{matrix}\ 1\right] {}_2F_1\left[\begin{matrix} m_2-k_2, m_2-k_2+t+1 \\ 2m_2+1 \end{matrix}\ 1\right]$$

Where s and t take all integral values from 0 to ∞

(ii) $\sum^{\infty} U\ (x,y) \frac{k_1-\frac{1}{2}}{m_1} \quad \frac{k_2+\frac{1}{2}}{m_2} \rightarrow \sum_{\nu=0}^{\infty} \sum_{m=}^{\infty}$

$$\sum^{\infty} (-1)^{m+} \frac{\Gamma(m_1-k_1+\nu+2m+1)}{\lfloor m\ \Gamma(n+\nu+2m+1)\ p^{\nu+2m}}\ x$$

$$\frac{\Gamma m_2-k_2+2n+1}{q^{2n}} \quad {}_2F_1\left[\begin{matrix} m_1-k_1, m_1-k_1+\nu+2n_1+1 \\ 2m_1+1 \end{matrix}\quad 1\right]$$

$${}_2F_1\left[\begin{matrix} m_2-k_2\ m_2-k_2+2n+1 \\ 2m_2+1 \end{matrix}\quad 1\right]$$

Where $(2m_r+1) \angle 0 \quad r=1,2$

Some deductions are also made from these results.

5 In section V we have derived two important theorems —

Theorem I If $x\ y^n f(x,y) \frac{n+2s+\frac{1}{2}}{s} \quad \frac{n+2s+\frac{1}{2}}{s} \quad \phi_{n,s}(p\ q)$

where $f(x,y)$ is not a function of n, then

$$\sum_{n=0}^{\infty} \frac{(n-a+1)\ (pq\epsilon)}{\lfloor n\ \Gamma(a+1)\Gamma(a+1)} \phi_{n,n}(p\ q) = (pq)^{1-a/2} \ -a/2\ (1-\epsilon)^{-1}x$$

$$\int_0^{\infty}\int_0^{\infty} \exp\left[\frac{-\epsilon\ xp+yq}{1-\epsilon}\right](xy)^{-a/2}\ I_a\left[\frac{2(xy\ pq)^{\frac{1}{2}}}{1-\epsilon}\right] f(x,y)\ dx\ dy$$

Provided (i) R $(n_1+1)>$ $_1$ R$(n_2+1)>$ where

$f(x\ y)=0\ (x)$ for small x and
$f\ (x,y)=0\ (y^{n}_{2})$ for small y

(ii) $f(x,y)$ is a continuous function of x and y for
$x \geqslant \theta > \quad y \geqslant \eta > \quad |\epsilon| < 1$ $\text{Re}p \geqslant P_0 >$ $\text{Re}q \geqslant q^0 >$

(iii) Integral involved is absolutely convergent

Theorem II If $f(x\ y)$ $\dfrac{n+a/^{2}+\frac{1}{2}}{a/^{2}+\gamma}$ $\dfrac{n+a/^{2}}{a/^{2}+\gamma}$ $\phi_{n-r,n-r}(p\ q)$ then

$$\sum_{r=}^{n} (-1)^r \frac{\Gamma(a+2r)\ \Gamma(a+r)\ \Gamma(a+n+r+1)}{\lfloor\ \lfloor\ \Gamma(a+2r+1)\ \Gamma(a+2r+1)} \phi_{n-r\ n-r}(p\ q)$$

$$= pq \int_0^{\infty}\int_0^{\infty} (xypq)^{-n}\ e^{-xp-yq} \lfloor_{n}^{a}\ (xp+yq) f(x\ y)\ dx\ dy$$

Provided (i) R $(n_1+1)>o$ where $f(x\ y)=0(x^{n_1})$ for small x

(ii) R $(n_2+1)>o$ where $f(x,y)=0(y^{n}2)$ for small y

(iii) $f(x\ y)$ is continuous function of x and y for $x \geqslant \theta > o$, $y \geqslant \eta > o$ Re $p \geqslant p_0 > o$, Re $q \geqslant q_0 > o$

(iv) Integral involved is convergent.

Some examples have also been evaluated to illustrate their uses

Chapter 3

Generalized Laplace Transform in two variables

1 In section I we have introduced the generalized laplace transform in two variables as

$$\phi(p,q) = pq \int_0^{\infty}\int_0^{\infty} e^{-\frac{xp}{2} - \frac{yq}{2}} (xp)^{-k_1-\frac{1}{2}}\ (yq)^{-k_2-\frac{1}{2}}\ W_{k_1+\frac{1}{2},m_1}(xp)$$

$$M_{k_2+\frac{1}{2},m_2}(yq)\ f(x,y)\ dx\ dy$$ which is symbolically denoted as

$$f(x,y) \xrightarrow[m_1]{k_1+\frac{1}{2}} \xrightarrow[m_2]{k_2+\frac{1}{2}} \phi(p,q)$$

From it the following deductions have also been made —

(i) $\phi\ (p\ q) = pq \int_0^\infty \int_0^\infty e^{-xp-yq} f(x,y)\, dx\, dy$ where $k_1 = m_1,\ k_2 = m_2$

(ii) $\phi\ (p,q) = 2^{2m_2 - n/2}\ \Gamma(m_2+1)\ pq \int_0^\infty \int_0^\infty e^{\frac{xp}{2} - \frac{yq}{2}} (xp)^{-n/2}\ (yq)$

$D_n(2xp)^{\frac{1}{2}}\ I_{m_2}\ \frac{yq}{2} f(x,y)\, dx\, dy$

when $k_1 = \frac{n}{2} - \frac{1}{4},\ m_1 = \frac{1}{4},\ k_2 = -\frac{1}{2}$

(iii) $\phi\ (p,q) = 2^{2m_2}\ \Gamma(2m_2+1)\ pq \int_0^\infty \int_0^\infty e^{\frac{xp}{2} - \frac{yq}{2}} (xp)^{\frac{1}{2}}\ (yq)^{\frac{1}{2}}$

$K_{m_1}\left(\frac{xp}{2}\right) I_{m_2}\left(\frac{yq}{2}\right) f(x\ y)\ dx\ dy$

where $k_1 = k_2 = -\frac{1}{2}$

(iv) $\phi\ (p\ q) = (-1)^{n_1}\ \frac{\lfloor n_1 \ \lfloor n_2\ \Gamma(2m_2+1)}{\Gamma(2m_2+1+n_2)}$

$pq \int_0^\infty \int_0^\infty e^{-xp-yq}\ (xp)^{-m_1} (yq)^{-m_2}\ L_{n_1}^{2m_1}(xp)\, L_{n_2}^{2m_2}(yq)\, f(x,y)$

$dx\ dy$

where $k_1 = m_1 + n_1\ \ k_2 = m_2 + n_2$

The images $\phi(p\ q)$ of the following originals $f(x,y)$ have also been evaluated.

(i) $(xy)^{\frac{\nu}{2}}\ J\left(\frac{2}{xy}\right)^{\frac{1}{2}}$ (ii) ${}_mF_n\left[\begin{matrix} a_1, a_2 \\ \beta_1\ \beta_2 \end{matrix}\ \ \beta_n^{n}\ -\frac{x^n}{y^n}\right]$

(iii) $\text{Sin}\ (x/y)^{\frac{1}{2}}$ (iv) $(x/y)^{-\lambda}\ J_{\lambda+}\ (x/y)^{\frac{1}{2}}\ J_{\lambda-}\ (xy)^{\frac{1}{2}}$

(v) $e^{-\frac{y}{2x}} W_{\lambda,\nu}\ (y/x)$

2. In section II we have established two theorems and some corollaries are discussed to illustrate their uses.

Theorem I. If $f(x\ y)\ \frac{k_1+\frac{1}{2}}{m_1}\ \ \frac{k_2+\frac{1}{2}}{m_2}\ \phi(p\ q)$ then

$$\phi(p\ q) = \frac{2\Gamma(m_2+\frac{1}{2})}{\Gamma(m_2-k_2)\ \Gamma(\frac{1}{2}+k_2)\ \Gamma(\frac{1}{2}-k_1)}$$

$$\int_0^\infty\int_0^\infty e^{-s}\, s^{-k_1-1}\, t^{k_2}\, \phi(p\, q, s\, t)\; ds\; dt$$

where $\phi(p, q \quad s, t) = pq \int_0^\infty\int_0^\infty e^{-\frac{xp}{2}} (xp)^{-k}(yq)^{-k_2}\, K_{2m_1}\left[2(xps)^{\frac{1}{2}}\right]$

$J_{m_2}\left[2(yqt)^{\frac{1}{2}}\right] f(x\, y)\; dx\; dy$

Theorem II If $x^{n} y^{n} f(x\, y)\ \frac{n+a/2+\frac{1}{2}}{a/2}\quad \frac{n+a/2+\frac{1}{2}}{a/2} \rightarrow \underset{n\ n}{\phi}(p, q)$

where $f(x, y)$ is not a function of n then

$$pq \int_0^\infty\int_0^\infty (xypq)^{-a}\, \Gamma(a, xp)\, \gamma(n\ \ yq)\, f(x, y)\; dx\; dy = \sum_{n=0}^{\infty} \frac{\Gamma(a)}{\Gamma(a+1)}$$

$$\frac{(-1)^n}{\lfloor(n+1)}(pq)\quad \underset{n\ n}{\phi}(p\ q)$$

where $\Gamma(a, x) = \int_x^\infty \ \ ^{-t}\, t^{a-1}\, dt\ ;\ a >$

$\gamma\ (a, x) = \int^x e^{-t}\, t^{a-1}\, dt \quad a >$

Provided (i) Re $(n_1+1) >$ where $f(x\ y) = 0(x \quad)$ for small x

(ii) Re $(n_2+1) >$ where $f(x\ y) = 0(y\ _n)$ for small y

(iii) $f\ (x, y)$ is a continuous function of x and y for $x \geqslant$ $G >\ \ y \geqslant \eta >^{o}$ Re $(p) \geqslant p^{o} >_{o}$, Re $(q) \geqslant q^{o} >$

(iv) Integrals involved are convergent

Chapter 4

On Meijer Transforms of three Variables

In section I we have introduced the Meijer Transform in three variables as

$$\phi(p\ q, r) = pqr \int_0^\infty\int_0^\infty\int^\infty -\frac{xp}{2} - \frac{yq}{2} - \ \overline{2}\ (xp)^{-k-\frac{1}{2}}\ (yq)^{-k_2-\frac{1}{2}}$$

$$(\ z)^{-k_3\ \frac{1}{2}}$$

$$W^{(xp)}_{k_1+\frac{1}{2}\ m_1}\ W^{(yq)}_{k_2+\frac{1}{2}\ m}\ W^{(rz)}_{k+\frac{1}{2}\ m_3}\ f(x\ y\ z)\; dx\; dy\; dz$$

Which is symbolically denoted as

$$f(x, y, z)\ \frac{k_1+\frac{1}{2}}{m_1}\quad \frac{k_2+\frac{1}{2}}{m_2}\quad \frac{k_3+\frac{1}{2}}{m_3} \rightarrow \phi(p\ q, r)$$

The following deductions have also been made from it

(i) $\phi(p,q,r)=pqr\int_0^\infty\int_0^\infty\int_0^\infty e^{-xp-yq-r} f(x,y\ )\,dx\,dy\,d$, when $k_1=m_1, k_2=m_2, k_3=m_3$

(ii) $\phi(p\ q\ r)=2^{-\frac{n_1+n_2+n_3}{2}}pqr\int_0^\infty\int_0^\infty\int_0^\infty e^{-\frac{xp}{2}-\frac{yq}{2}-\frac{r}{2}}$

$(xp)^{\frac{-n}{2}}(yq)^{\frac{-n_2}{2}}(rz)^{\frac{-n}{2}}$ ...

$D_1[(2xp)^{1/2}]\ D_2[(2yq)^{1/2}]\ D_3[(2rz)^{1/2}]f(x,y,\ )\,dx\,dy\,d$

where $k_1=\frac{n_1}{2}-\frac{1}{4}$ $k_2=\frac{n_2}{2}-\frac{1}{4}$ $k_3=\frac{n}{2}-\frac{1}{4}$ $m_1=m_2=m_3=\frac{1}{4}$

(iii) $\phi\ (p\ q\ r)=(-1)^{l_1+l_2+l_3}\lfloor l_1\ \lfloor l_2\ \lfloor l_3\ pqr\int_0^\infty\int_0^\infty\int e^{\dots}$

$(xp)^{-l_1}\ (yq)^{-l_2}\ (rz)^{-l_3}$

$\lfloor^{n_1}_{l_1}(xp)\ \lfloor^{n_2}_{l_2}(yq)\ \lfloor^{n_3}_{l_3}(r\ )\ f(x,y,\ )\ dx\ dy\ d$ where $k_1=l_1+\frac{n_1}{2}$

$k_2=l_2+\frac{n_2}{2}$ $k_3=l_3+\frac{n_3}{2}$ $m_1=\pm\frac{n_1}{2}$ $m_2=\pm\frac{n_2}{2}$ $m_3=\pm\frac{n}{2}$

(iv) $\phi(p\ q,r)=-\frac{pqr}{\pi\sqrt{\pi}}\int_0^\infty\int_0^\infty\int_0^\infty e^{\frac{xp}{2}-\frac{yq}{2}-\frac{r}{2}}(xp)^{\frac{1}{2}}\ (yq)^{\frac{1}{2}}\ (r\ )^{\frac{1}{2}}$

$K_{m_1}\left(\frac{xp}{2}\right)K_{m_2}\left(\frac{yq}{2}\right)K_{m_3}\left(\frac{r}{2}\right)$

$f\ (x,y,z)\ dx\ dy\ d$ where $k_r=-\frac{1}{2}$ $r=1,2,3$

(v) $\phi\ (p\ q\ r)$

$=pqr\int^\infty\int^\infty\int^\infty \frac{[E\ n_1-l_1\ \ xp]\ E\ [n_2-l\ \ \ yq]\ E\ [n_3-l_3\ \dots]}{\Gamma(n_1)\ \Gamma(n_2)\ \Gamma(n_3)\ \Gamma(l\ )\ \Gamma(l_2)\ \Gamma(l_3)}$

$f(x\ \ y\ \ \ )\ dx\ dy\ d$

Where E-function is MacRobert's function.

Then we have obtained the images $\phi(p\ q)$ corresponding to the f ... originals $f\ (x\ )$

(i) $x\ y^m$ (ii) $(x+y+\ )$ (iii) $xy\ )\ ^{\mu}\ J\ [^{\nu}(xy\ )^{\frac{1}{2}}]$

(iv) $(xy\ )^{-\frac{1}{2}}$ H $[2(xy\ )^{\frac{1}{2}}]$ (v) $(xy\ )\ ^{\mu}$ I $[^{\nu}(xy\ )^{\frac{1}{2}}]$

(vi) J $[2(xy\ )^{\frac{1}{2}}]$ J $[2(x\ y)^{\frac{1}{2}}]$ $(xy\ )^{\frac{n+r}{2}}$

2 In section II we have established the following important t ...

Theorem I. If $\phi(p,q,r)_{m-n-s,\,m-n-s,\,m-n} \doteq \underset{m-n-s}{\overset{a+2n+2s}{L}}(xp)$

$\underset{m-n-s}{\overset{a+2n+2s}{L}}(yq) \quad \underset{m-n}{\overset{a+2n}{L}}(rz)-$

Transform of $f(x,y,z)$, then

$$pqr\int_0^\infty\int_0^\infty\int_0^\infty (xyzpqr)^{-m} \underset{m}{\overset{a}{L}} (xp+yq+rz)\ f(x,y,z)\ dx\,dy\,dz$$

$$=\sum_{n=0}^{m}\ \sum_{s=0}^{m-n} (-1)^{-m+2n+s}$$

$$\frac{\Gamma(a+2n)\ \Gamma(a+n)\ \Gamma(a+2n+2s)\ \Gamma(a+s+2n)}{\lfloor n\ \lfloor s\ \lfloor m-n-s\ \Gamma(a+m+n+1)\ \Gamma(a+m+n+s+1)}\ \phi(p,q,r)_{m-n-s,\ m-n-\ \ -s,\ m-n}$$

Provided (i) $\mathrm{Re}(n_1+1)>0$, $\mathrm{Re}(n_2+1)>0$, $\mathrm{Re}(n_3+1)>$ where $f(x,y,z)=0(x^{n_1})$ for small x, $f(x,y,z)=0(y^{n_2})$ for small y, $f(x,y,z)=0(z^{n_3})$ for small z.

(ii) $f(x,y,z)$ is a continuous function of x,y,z for $x\geqslant\epsilon>0$, $y\geqslant\eta>0$, $z\geqslant\xi>$

(iii) $\mathrm{Re}\,p\geqslant p_0>0$, $\mathrm{Re}\,q\geqslant q_0>0$, $\mathrm{Re}\,r\geqslant r_0>$

(iv) Integral involved is convergent

Theorem II. If $\phi(p,q,r)\ \frac{n+\frac{1}{2}+a/2}{+a/2}\ \ \frac{n+a/2+\frac{1}{2}}{+a/2}$

$$\frac{\frac{n}{2}3+l_3+\frac{1}{2}}{+\frac{n_1}{2}}\ (xy)\quad f(x,y,z)$$

then $\sum_{n=0}^{\infty} \frac{t^n\ (qp)}{\lfloor n\ \Gamma(n+a+1)}\ \phi(p,q,r)=(-1)^{l_3}\lfloor l_3\times$

$$pqr\int_0^\infty\int_0^\infty\int_0^\infty e^{-\frac{xp}{2}-\frac{yq}{2}-rz}$$

$$\frac{(xypqt)^{-a/2}}{1-t}\ \mathrm{Exp.}\left[-\tfrac{1}{2}(xp+yq)\ \frac{1+t}{1-t}\right] I\left[\frac{2(xypqt)^{1/2}}{1-t}\right](zr)^{-l_3}$$

$L_{l_3}(rz)\quad f(x,y,z)\ dx\,dy\,dz$

Provided (i) the integrals are absolutely convergent and $f(x,y,z)$ is not a function of n (ii) $f(x,y,z)=0(x^{n_1})$ for small x, $\mathrm{Re}(n_1+1)>0$, $f(x,y,z)=0(y^{n_2})$ for small y, $\mathrm{Re}(n_2+1)>0$, $f(x,y,z)=0(z^{n_3})$ for small z, $\mathrm{Re}(n_3+1)>0$ (iii) $\mathrm{Re}\,p\geqslant p_0>0$, $\mathrm{Re}\,q\geqslant q_0>0$, $\mathrm{Re}\,r\geqslant r>$

Theorem III. If $z^{m/2}(xy)^{n/2} f(x\ y\ z) \frac{n/2+\frac{1}{2}}{+\frac{1}{2}}\ \frac{n/2+\frac{1}{2}}{\frac{1}{2}}\ \frac{n/2+\frac{1}{2}}{\frac{1}{2}} \phi(p\ q\ r)$

where $f(x\ y\ z)$ is not a function of n, then

$$\sum_{n=0}^{\infty} \frac{(2t)^n (pq)^{n/2} r^{m/2}}{\lfloor n} \phi_{n,n,m}(p\ q\ r) = \sqrt{\frac{2}{\pi}}$$

$$pqr \int_0^\infty \int_0^\infty \int_0^\infty e^{\frac{1}{2}(xp+yq+2rz)x} \frac{1}{1-t^2}$$

$$exp \left[\frac{(xypq)^{\frac{1}{2}} - (xp+yq)\quad (1+t^2)}{1-t^2} \right] e^{-\frac{s^2}{2}}$$

$$s^m \cos \left[s(2rz)^{\frac{1}{2}} - \frac{m\pi}{2} \right] dx\ dy\ dz$$

Theorem IV If $x\ y\ z^m f(x\ y\ z)\ \frac{n}{\frac{1}{2}}\ \frac{n}{\frac{1}{2}}\ \frac{m}{\frac{1}{2}} \rightarrow \phi_{n-\frac{1}{2}\ n-\frac{1}{2}\ m-\frac{1}{2}}(p\ q\ r)$ where

$f(x\ y\ z)$ is not a function of n, then

$$\sum_{n=1}^{\infty} \frac{n\,(pq)^{n\ 1}\ t^{n-1}}{(\lfloor n)^2} \phi_{n-\frac{1}{2}\ n-\frac{1}{2}\ m-\frac{1}{2}}(p\ q\ r) = r^{-m} \lfloor m$$

$$pqr \int^\infty \int^\infty \int^\infty e^{\frac{1}{2}(xp+yq+rz)}\ x$$

$$K_{2m}\left(\frac{rz}{2}\right) (xypq)^{\frac{1}{2}} \frac{t^{\frac{1}{2}}}{1-t}\ exp \left[-(xp+yq)\ \frac{1+t}{1-t} \right]$$

$$I_1 \left[\frac{2(xy-pq)}{1-t} \right] f(x\ y\ z) \qquad dx\ dy\ dz$$

where $|t| < 1$ and integrals and series involved are convergent

Theorem V If (i) $(s,\ t\ w)^{-n+\frac{1}{2}} f(s\ t\ w)\ \frac{k_1+\frac{1}{2}}{m_1}\ \frac{k_2+\frac{1}{2}}{m_2}$

$$\frac{k+\frac{1}{2}}{m_3} \rightarrow \phi_n(p\ q\ r) \text{ and}$$

(ii) $\psi(p\ q\ r) = e^{\frac{1}{2}(sp+tq+rw)} s^{-(n+k_1)}$

$$t^{-(n+k_2)}\ w^{-(n+k_3)}$$

$$W_{k_1+\frac{1}{2}\ m_1}(sp)\ W_{k_2+\frac{1}{2}\ m_2}(qt)\ W_{k_3+\frac{1}{2}\ m_3}(r\ )$$

$f(s\ t\ w)$ then

$$\phi_{n+l}(p\ q\ r) = \frac{p^{-k_1+\frac{1}{2}}\ q^{-k_2+\frac{1}{2}}\ r^{-k_3+\frac{1}{2}}}{(\pi)^{3}}$$

$$\int_0^\infty \int_0^\infty \int_0^\infty \psi(x\ y\ z)\ (xy\ )^{1-n}\ dx\, dy\, d$$

provided the integrals involved are convergent and Meijer's Transform of $(stw)^{\frac{1}{2}-n} f(s\ t\ w)$ exists.

Chapter 5

Some properties of Laplace Transform of two variables

1 In section I two rules have been obtained and some examples have been solved to show their applications

Rule I. If $\phi(p\ q) = pq \int_0^\infty \int_0^\infty e^{-xp-yq} f(x\ y)\ dx\ dy$ then

$$\frac{\phi\left(\frac{1}{p}\ \frac{1}{q}\right)}{(pq)^{v-1}} = \int_0^\infty \int_0^\infty \left(\frac{xy}{sy}\right)^{v/2} J\ (2\sqrt{xs})\ J\ (2\sqrt{yt})\ f(s,\ t)\ ds\ dt$$

where Re $p \geq p_0 > 0$ Re $q \geq q_0 > 0$ Re $v > -1$ Re $(pq) > 0$

Rule II If $f(x\ y) = \phi(pq)$ then

$$f(e^{x}-1\ \ e^{y}-1) = \frac{1}{\Gamma(p)\Gamma(q)} \int_0^\infty \int_0^\infty e^{-(u+v)}\ u^{p-1}\ \phi(u,\ v)\ du\ d$$

provided the image of $f(e\ -1\ \ e^{y}-1)$ exists and the integrals involved are absolutely convergent.

2. In section II we have obtained two important theorems.

Theorem I. If $\phi(p\ q) = p\ q \int_0^\infty \int_0^\infty e^{-xp-yq} f(x\ y)\ dx\ dy$ and

$$x^{m-k+\frac{1}{2}} f\left(\frac{1}{x}\ \frac{1}{y}\right) y^{m_1-k_1+\frac{1}{2}} = xy \int_0^\infty \int_0^\infty e^{-\frac{xu}{2}-\frac{yv}{2}}$$

$W_{k,\ m}(x\ u)$

$W_{k_1\ m_1}(yv)\ (xu)^{m-\frac{1}{2}}\ (yv)^{m_1-\frac{1}{2}}\ \phi(u,\ v)\ du\ dv$ then

$$\phi(p\ q) = 2p^{k+\frac{1}{2}}\ q^{k_1+\frac{1}{2}} \int_0^\infty \int_0^\infty u^{m}\ v^{m_1}\ K_{2m}\ [2(up)^{\frac{1}{2}}]$$

$K_{2m_1}[2\ (vq)^{\frac{1}{2}}]\ \phi(u,\ v)\ du\, dv$

Provided Re $(p)>o$ Re $(\mu_1+1)>o$ Re $(2m+\mu_1+1)>o$
Re $(q)>o$ Re $(\nu_1+1)>o$ Re $(2m_1+\nu_1+1)>o$ where
$\phi(u, v)=o(u^{\mu_1})$ for small u and
$\phi(u\ v)=o(v^{\nu_1})$ for small v and
the integrals are absolutely convergent

Theorem II. If $f(p) \doteq h(x)$ imply $ph(p) \doteq x^{\frac{1}{2}} g(\ )$

then (i) $f(p)=p\int_0^{\infty} \frac{t^{\frac{1}{2}}g(t)}{p+t}\,dt$

(ii) $f(4pq)=4pq\int_0^{\infty} \frac{t^{\frac{1}{2}}g(t)}{4pq+t}\,dt$

(iii) $\int_0^{\infty} g(t)\,(xt)^{-\frac{v}{2}}\ \frac{v}{y^2}\,H\ (xyt)^{\frac{1}{2}}\,dt \doteq \frac{f(4pq)}{2^{v+3}\ (pq)^{3+v}}$

where H (x) is struve's Function

3 In section III we have obtained the following transforms

(i) $y\ U_v(x\ y) \doteq \sum_{m=}^{\infty} (-1)^m\, \Gamma(1+v)\,\Gamma(1+v+2m)$

$$r^{2m-1}\ P_{-2m}^{-v-2m}(p/r)$$
$$p^{v+2m-1}$$

where $r=(p^2+1)^{\frac{1}{2}}$ Re $(v)>-1$

where U $(w\ z)=\sum_{m=}^{\infty} (-1)^m \left(\frac{w}{z}\right)^{v+2m} J_{v+2m}(\ )$ Lommel's Functions of two variables

(ii) $(1-x)^{-\alpha}\ (1-y)^{-\beta} \doteq \sum_{n=}^{\infty} \sum_{r=}^{\infty} \frac{(\alpha)_r\ (\beta)_{n-r}}{p^r\ q^{n-r}}$

(iii) $x^{\beta-1}\,E_{\alpha\ \beta}\ (x^{\alpha}y) \doteq \frac{1}{\beta-1}\ {}_2F\left[1\ 1\ \frac{1}{p^{\alpha}q}\right]$

where $E\alpha\ \beta(\ )=\sum_{k=}^{\infty} \frac{z^k}{\Gamma(\alpha k+\beta)}$

(v) $x^{\beta-1}\ \phi\ (\alpha, \beta\ x^{\alpha}y) \doteq \frac{p^{\alpha-\beta+1}}{p^{\alpha}q-1}$

where $\phi(\ \ \beta\ z)=\sum_{k=}^{\infty} \frac{z^k}{\lfloor k\ \Gamma(\alpha k+\beta)}$

Chapter 6

On Symbolic Calculus of two variables

1 In section I we have derived some new results and some integrals have been evaluated to show their applications

Result I. If (i) $f(p) \doteq x^{\nu-1} h(x)$ imply $h(p) \doteq g(x)$

$$(ii)\quad p^{n-\lambda}\, h\left(\frac{1}{p^{n}}\right) \doteq \sigma(x)$$

(iii) $f_1(x) \frac{m+\frac{1}{2}}{m} \phi(p)$ and $f_1(x) \doteq J(hx)$ then

$$y^{-\frac{\lambda}{n}-\frac{1}{2}}\, \sigma\left(xy^{-\frac{1}{n}} h^{\frac{2}{n}}\right) \doteq \frac{\sqrt{\pi q}}{p\lambda}\, h^{\frac{2\lambda}{4}} \int^{\infty} g(t)\phi\left(\frac{p^{\frac{n}{2}}\, q^{\frac{1}{2}}}{t^{\frac{1}{2}}}\right)dt$$

Result II If (i) $f(p) \doteq x^{\nu-1} h(x)$ imply $h(p) \doteq g(x)$

$$(ii)\quad p^{n-\lambda}\, h\left(\frac{1}{p}\right) \doteq \sigma(x)$$

(iii) (a) $f_1(x) \frac{m+\frac{1}{2}}{m} \rightarrow \phi(p)$ and $f(x) \doteq e^{-}/^{4}\, D_0(\sqrt{x})$ then

$$y^{-\lambda/}\, \sigma(xy^{1}/\ ) \doteq \frac{1}{p\lambda} \int_{0}^{\infty} g(t)\, \phi\left(\frac{p\, q}{2t}\right)dt$$

(b) $f_1(x) \frac{m+\frac{1}{2}}{m} \phi(p)$ and $f(x) \doteq e^{-x/4}\, D_1(\sqrt{x})$ then

$$y^{\frac{1}{2}-\lambda/}\, \sigma(xy)\, / \doteq p^{n}/^{2-\lambda} \int^{\infty} \frac{1}{\sqrt{2t}}\, g(t)\, \phi\left(\frac{p^{n} q}{2t}\right)dt$$

Result III.—If (i) $f(p) \doteq x^{\nu-}\ \sigma(x)$ imply $h(p) \doteq g(x)$

$$(ii)\quad p^{n-\lambda}\, h\left(\frac{1}{p^{n}}\right) \doteq \sigma(\ )$$

(iii) $f(x) \frac{m+\frac{1}{2}}{m} \rightarrow \phi(p)$ and $f(x) \doteq {}^{-}$ then

$$y^{-\lambda/n}\, \sigma(xy^{1}/\ ) \doteq p^{-\lambda} \int_{0}^{\infty} g(t)\phi\left(\frac{p\, q}{t}\right)dt$$

2. In section II we have established a theorem

Theorem If (i) $f_2 \; (x) \; \frac{k}{m} \rightarrow f_1 (p)$

(ii) $p^{1-\lambda} + f_2 (p)$ ——— $g(t)$ then

$$\sum_{r=0}^{\infty} \frac{(-1)^r \; \Gamma\left(\frac{\lambda}{v}+\frac{\gamma}{v}+\frac{1}{4} \pm m\right)}{\lfloor \gamma \; \Gamma\left(\frac{\lambda}{v}+\frac{\gamma}{v}+\frac{3}{4}-k\right)} \frac{x^{\frac{\alpha}{v}(\lambda+\gamma)} \; y^{\frac{\beta}{v}(\lambda+\gamma)}}{\Gamma\left(\frac{\alpha\gamma}{v}+\frac{\alpha\lambda}{v}+1\right) \; \Gamma\left(\frac{\beta\gamma}{v}+\frac{\beta\lambda}{v}+1\right)}$$

$$\int^{\infty} \frac{s^{\gamma} g\,(s)}{s^{(\lambda+\gamma)/v}} \; {}_2F_1 \left[\begin{matrix} \frac{\lambda}{v}+\frac{\gamma}{v}+\frac{1}{4} \pm m \\ \frac{\lambda}{v}+\frac{\gamma}{v}+\frac{3}{4}-k \end{matrix} \; \right] ds \; ———$$

$$f_1 (p^{\alpha} q^{\beta}) / p \; q^{\beta}$$

Provided the integrals involved are convergent. Some examples have been evaluated to illustrate its application

Chapter 7

SOME PROPERTIES OF HANKEL'S TRANSFORMS

1 We have introduced the transforms —

(i) $\phi \; (x) = \int^{\infty} \int^{\infty} (xy)^{\frac{1}{2}} \; J \; (xy) \; J \; (x \;) f(y \;) \, dy \, d$

where $f(y, \;)$ is a function of two variables y and

(i) $\phi \; (x \; y) = \int^{\infty} \int^{\infty} (xs)^{\frac{1}{2}} \; J \; (xs)(yt)^{\frac{1}{2}} \; J \; (yt) \, f(s,t) \; ds \; dt$

as the generalizations of the well known Hankel's Transform of order

$$\phi \; x) = \int^{\infty} xy)^{\frac{1}{2}} \; J \; (xy) \, f(y) \, dy$$

(a) We have also obtained $\phi \quad)$ when $f(y \;)$ is given of the f m king use of the transform (i)

(i) $(y \quad + \frac{1}{2} \; (y^2 + \;)^{\rho-r-1} \; y^{-\rho}$ (ii) $e^{-ry}(y \;)^{\rho-\gamma} \; U \; (y \;)$

(iii) $e^{(\rho v \quad r)} \; (\; y)^{\lambda-3/2} J \; (y \;) \quad (\;) \quad e^{-y} \; \rho - \frac{1}{2}$

(v) $z^{\frac{1}{2}} J(b)(z^2+y^2)^{-1}$ $y^{v+\frac{1}{2}} J(yz)(z^2+\beta^2)^{-1} z^{u-v+\frac{1}{2}} y^{u+v+1}$

(vi) $J(y) e^{y^2-rz} z^{\lambda-3/2}$ (vii) $S_{u,v}(z)$ $z^{u+\frac{1}{2}} y^{v-\frac{1}{2}}$

(b) Further we have obtained $\phi(x\ y)$ where $f(s\ t)$ is given making use of (ii) of the following originals.

(i) s^{1} t^{n} (ii) $(st)^{-}$ $J_v(st)$

2 In section II we have obtained the following theorems —

Theorem I. If $f(x\ y)\ \dfrac{k_1+\frac{1}{2}}{m_1}\ \ \dfrac{k_2+\frac{1}{2}}{m_2} \rightarrow \phi(p\ q)$ and

$x^{2}\ ^{-3/2}\ y^{2}\ _{2}^{-3/2}\ x\ \phi(x^2, y^2)$ is self reciprocal in Hankel's Transform of order V_1 and V_2, then

$$\int_0^{\infty}\int_0^{\infty} \sum_{m=0}^{\infty} \sum_{n}^{\infty} (-1)^{m+n}$$

$$\frac{\Gamma(m_1-k_1+n_1+v_1/_2+m+1)\ \Gamma(m_2-k_2+n_2+v_2/_2+n+1)\ (a/2)_{1}^{+2m}}{\Gamma(m+v_1+1)\ \Gamma(v+n+1) \lfloor m \lfloor\ x^{\ 1+\ _1/_2+m+1}\ y^{\ 2+\ _2/_2+n+1}}$$

$$(b/2)_{2}^{+2n}\ {}_2F\left[\begin{matrix} m_1-k_1\ \ m_1-k_1+n_1+v_1/_2+1+m \\ 2m_1+1 \end{matrix}\ \ 1\right]$$

$$ {}_2F_1\left[\begin{matrix} m_2-k_2,\ m_2-k_2+n_2+v_2/_2+n+1 \\ 2m_2+1 \end{matrix}\ \ 1\right] f(x,y)\ dx\ dy =$$

$$\left[2a_{1}\ b_{2}^{1}\right]^{2} \phi(a^2\ b^2)$$

Provided Re $(n_r+v_r/2+1-k_r) > |$ Re $m_r|$ $r=1$ 2 and $f(x,y)$ is continuous function of x and y for $x \geqslant \theta > 0$, $y \geqslant \eta >$ and integrals are absolutely convergent.

Theorem II. If $f(x,y)\ \dfrac{k_1+\frac{1}{2}}{m_1}\ \dfrac{k_2+\frac{1}{2}}{m_2} \rightarrow \phi(p\ q)$ and $x^{n_1-3/2}\ y^{\ \ 3/2}\ \phi$ (x,y) is self reciprocal function in Hankel's Transform of order V and V_2 then

$$\int_0^{\infty}\int_0^{\infty} \sum_{m=}^{\infty} \sum_{n=}^{\infty}$$

$$\frac{(-1)^{m+n}\ \Gamma(m_1-k_1+n+v_1+2m+1)\ \Gamma(m_2-k_2+n_2+v_2+2n+1)}{\lfloor m \lfloor\ \Gamma(v_1+m+1)\ \Gamma(v_2+n+1)\ x^{n_1+v_1+2m+1}\ y^{n_2+v+2n+1}} (a/2)_{1}^{+2m}$$

$$(b/2)_{2}^{+2n}\ {}_2F_1\left[\begin{matrix} m_1-k_1,\ m_1-k_1+n+v+2m+1 \\ 2m_1+1 \end{matrix}\ \ 1\right]$$

$$ {}_2F_1\left[\begin{matrix} m_2-k_2,\ m_2-k_2+n_2+v+2n+1 \\ 2m_2+1 \end{matrix}\ \ 1\right] f(x\ y)\ dx\ dy$$

$$= a^{n_1-2}\ b^{n_2-2}\ \phi(a, b)$$

Provided Re $(n_r+v_r+1-k_r) > |$ Re $m_r|$ $r=1$ 2 and $f(x\ y)$ is a continuous function of x and y for $x \geqslant \theta > 0$ $y \geqslant \eta > 0$ and integrals involved are convergent.

Theorem III. If $f(x\ y) \doteq \phi\ (p\ q)$ and $x^{n_1 - r_1 - 3/2}\ y^{n_2 - r_2 - 3/2}$

$f\left(\frac{1}{x}\ \ \frac{1}{y}\right)$ is self reciprocal in the Hankel's Transform of order r_1 and r_2, then

$$(a/2)^{r_1} (b/2)^{r_2} \int_0^{\infty} \int_0^{\infty} x^{n_1 - 2}\ \ y^{n_2 - 2}$$

$$_0F_2\left[r_1+1\ \ \frac{n_1}{2}\ \ \frac{n_1}{2}+\tfrac{1}{2}\ \ -\frac{a^2 x^2}{16}\right]$$

$$_0F_2\left[r_2+1\ \ \frac{n_2}{2}\ \ \frac{n_2}{2}+\tfrac{1}{2}\ \ -\frac{b^2 y^2}{16}\right] \phi\ (x\ y)\ dx\ dy$$

$$= \frac{\Gamma(r_1+1)\ \Gamma(r_2+1)\ \Gamma(n_1)\ \Gamma(n_2)}{a^{r_1 - n_1 + 2}\ b^{r_2 - n_2 + 2}}\ f\,(1/a\ \ 1/b) \text{ where}$$

Re $(r - n_r + 1) > 0$ $r = 1, 2$ x $f(xy)$ is a continuous function of x and y for $x \geqslant \epsilon > 0$ $y \geqslant \eta > 0$ and integrals involved are absolutely convergent.

3 In section III we have discussed the following integrals involving Bessel's functions.

$$(i)\ \int^{\infty}\int^{\infty} (xy)^{\frac{1}{2}}\ J\ (xy)\ J\ (xz)\ x^{\frac{v+1}{2}}\ y^{\frac{v+3/2}{2}}\ \ \frac{x}{}+\tfrac{1}{2}\ e^{-n^2}$$

$$Sin\ h\left(\frac{x}{4y}\right)\ dy\ d$$

$$(i)\ \int_0^{\infty}\int^{\infty} J\ (xy)\ J\ (xy)\ J\ (b\ )\ S_n\ \ (r\ )\ x^{\rho - 1}\ \epsilon^{\lambda}\ y^{-\sigma}$$

$$dr\ d$$

$$(ii)\ \int_0^{\infty}\int_0^{\infty} (xy)^{\frac{1}{2}}\ J\ (xy)\ J\ (x\ )\ J\ (xy)\ \epsilon^{\frac{a^2 x^2\ 1}{4}}\ (y)^{\frac{1}{2}}\ dr\ d.$$

Chapter 8

On Some Results Involving Legendre Polynomials

Here we have obtained the following results.

$$(i)\ \ \int_{-1}^{1} 2^{n}\ \ \ (1-)^{n/}\ \ \sum_{k=0}^{n} C_{n,k}(-1)^{n-k}\left[\Gamma(1+x)\right]^{-\frac{n-k}{}}$$

$$P_k\left(\frac{1+x}{}\right)^{k} dx$$

$$= \frac{\pi^{\frac{1}{2}}}{\Gamma(1-n/2)} \quad \frac{1}{\Gamma(n/2+3/2)}$$

(ii) $$\int_{-1}^{1} 2^{n/2} \quad (1+x)^{n/2} \sum_{k=0}^{n} C_{n,k} \quad (-1)^{n-k}[2(1+x)]^{\frac{-n-k}{2}}$$

$$P_k \left(\frac{1+x}{2}\right)^{\frac{1}{2}} P_{\sigma}(x)\, dx$$

$$= \frac{4}{\pi} \quad \frac{\sin (\sigma-n)}{(\sigma-n)(\sigma+n+1)}$$ where $\sigma \neq n$ and σ is not an integer

$=0$ where $\sigma \neq n$ and σ is an integer

(iii) $$\int_{-1}^{1} \sum_{k=0}^{n} C_{n,k} \quad (-1)^{n-k} P_k \;(1-2x^2)\; dx = \frac{\pi^{\frac{1}{2}}\,\Gamma(1+n)}{\Gamma(n+3/2)}$$

where n is an even integer

(iv) $$2^n \int_{-1}^{1} x^{n+1}\; P_n\;(x)\;\; J_0\,(r \cos \theta \sqrt{1-x^2})\;\; J_0\;(r \sin \theta\;\; x)\; dx$$

$$= \sum_{k=} ^{n} \sum_{s=}^{n} C_{n,k} \;\; (r)\;\; P_s\;\; (\cos 2\theta) \qquad \text{where} \quad s=k$$

(v) $$\int_{0}^{\pi} \sin \theta \cos \frac{\theta}{2} \; P_n\left(\cos \frac{\theta}{2}\right) e^{-\frac{i\phi}{2}}\left[\cos^2 \frac{\phi}{2} - \cos \quad \frac{\theta}{2}\right]^{-\frac{1}{2}} d\theta$$

$$= \frac{1}{2n-2} \quad \sum_{k=}^{n} \quad \frac{e^k}{(2k+1)}\, C_{n,k} \qquad\qquad 0 \leq k \leq n$$

$$= 0 \qquad\qquad k > n$$

(vi) $$\int_{}^{\pi} Pn \left(\cos \frac{\theta}{2}\right) \text{Log} \left(\;1+\text{Cosec}\; \frac{\theta}{2}\right) \text{Sin}\; \theta \cos \;\; \frac{\theta}{2} \; d\,\theta$$

$$= \frac{1}{2^{n-1}} \quad \sum_{k=0} \; C_{n,k}/(k+1)\;\;(2k+1) \qquad\qquad 0 \leq k \leq n$$

$$= 0 \qquad\qquad k > n$$

(vii) (a) $$\int_{-1}^{1} \;(1+x)^{n/2} \quad P_n \left(\frac{1+x}{2}\right)^{\frac{1}{2}} \; e \;\; J \;\; (1-x^2)^{\frac{1}{2}} \; dx = \frac{1}{2^{n/2-1}}$$

$$\sum_{k=}^{n} \quad \frac{C_{n,\,k}}{\lfloor k \quad (2k+1)}$$

(b) $$\int_{-1}^{1} (1+x)^{n/2} \quad P_n \left(\frac{1+x}{2}\right)^{\frac{1}{2}} \;\; I_0\,[2\;(x-1)^{\frac{1}{2}}\;]\; I_0\;[2(x+1)^{\frac{1}{2}}\;] dx$$

$$= \frac{1}{2^{n/2-1}} \quad \sum_{k=}^{n} \quad \frac{C_{n,\,k}}{[\lfloor k\;]\;\;(2k+1)}$$

(viii) $$\int_{-1}^{1} (1+x)^{n/2} \; P_n \left(\frac{1+x}{2}\right)^{\frac{1}{2}} e^{xt} {}_2F_2 \; [\tfrac{1}{2} \; \tfrac{1}{2}; 1 \; \tfrac{1}{2} \; \tfrac{1}{2}; 1 - \tfrac{t^2}{4} (1-x^2)] \, dx$$

$$= \frac{1}{2^{n/2-1}} \sum_{k=0}^{n} \frac{t^k \; C_{n,k}}{\lfloor k \; (2k+1)}$$

(ix) $$\int_{-1}^{1} [2 \log 2 - 1 - 2 \log (1-t) (1-y)] \; (1+y)^{n/2} \; P_n \left(\frac{1+y}{2}\right)^{\frac{1}{2}} dy$$

$$= \frac{1}{2^{n/2-1}} \sum_{k=1}^{n} \frac{1}{k(k+1)} \; {}_2F_1 \; [-k, k+1 \;|\; \tfrac{1}{2}-t/2]$$

(x) $$\int_{-1}^{1} (1+x)^{n/2} \; P_n \left(\frac{1+x}{2}\right)^{\frac{1}{2}} (1+z^2-2zx)^{-\frac{1}{2}} \, dx$$

$$= \frac{1}{2^{n/2-1}} \sum_{k=0}^{n} \frac{z^k \; C_{n,k}}{(2k+1)}$$

(xi) $$\int_{-1}^{1} (1+x)^{n/2} \; P_n \left(\frac{1+x}{2}\right)^{\frac{1}{2}} x^k \; (1-x^2)^{\frac{1}{2}} \sum_{m=1}^{\infty} \frac{(-1)^{m-1} \Gamma(m-\frac{1}{2}) \; \Gamma(m+k) \; \Gamma(k+3)}{\Gamma(k+1) \; \Gamma(m) \; \Gamma(k+m+1)}$$

$$x^{m+1} \; (1-x^2)^{\frac{m-1}{2}} \, dx = \frac{\pi \lfloor (2k+1)}{2^{n/2+1+2k} \; [\lfloor k \;]^2 (2k+1)} C_{n,k}$$

(xii) $$\int^{\infty} \int^{1} y^{n+1} \; J_0 (xy)^n \; P \; (y) \, dy \, dx$$

$$= \frac{1}{2^{n+1}} \sum_{k=0}^{n} C_{n,k} \; (-1)^k \frac{1}{\Gamma 2k+1}$$

Apart from the results above stated some other results have also been obtained.

A STUDY OF MOTIONS IN ROTATING FLUIDS

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The motion of a body in a non viscous, incompressible rotating fluid was first studied by Proudman (1916) who remarked that a small steady three dimensional disturbance produced in a rotating fluid tended to become two-dimensional in character In the following years Taylor (1922, 1923) performed a series of experiments and discovered a number of interesting facts. He obtained a family of solutions to the non-linear equations for the case in which a sphere moves with a steady velocity along the axis of rotation of the fluid. In fact, there are not enough boundary conditions to obtain a unique solution Nigam has generalised Taylor's problem and Fadnis has obtained exact solutions of the Taylor's generalised problem in the following cases oblate and prolate spheroid, paraboloid of revolution, circular disc, a finite pressure line and a pressure point, and a semi-infinite pressure line In all these cases the steady state solution is not unique

Quite a considerable amount of work has been done in recent years on this problem by Gortler Morgan and Stewartson These authors have proved that the flow becomes steady and two-dimensional if the disturbance which causes it approaches a steady state This theoretical result is in agreement with the experimental results of Taylor except in the neighbourhood of the surface of an infinite cylinder which encloses the body whose generators are parallel to the axis of rotation.

The investigations reported in the thesis arose as a result of the study of the subject of Rotating Fluids The thesis deals with certain problems that arise when a three dimensional disturbance is introduced in a perfect, incompressible fluid otherwise rotating like a rigid body about an axis. In this work the fluid is assumed to be perfect It is characterised by one material constant, its density and all the flow phenomenon are governed by one non-dimensional parameter namely the Rossby number

The theory of rotating fluids has a mystery and fascination of its own and has attracted the attention of a large number of workers. However since 1916, a few difficulties have arisen which await a satisfactory theoretical explanation even today Work in this field has progressed along the following two lines

(i) *Exact steady-state solutions for the uniform motion of bodies of revolution along the axis of rotation* —

Taylor (1922) gave a solution of the non-linear equations

This is an abstract of the thesis submitted and approved for the Ph. D. degree of the Agra University for the year 1960.

for the case of a sphere which was not unique even when the boundary conditions of the perfect fluid theory were satisfied. This led to the following questions —

(*a*) What are the correct boundary conditions and whether they can be deduced from theoretical considerations?

(*b*) Whether the non-linear equations can be solved for any body of revolution?

(*c*) Whether the exact steady-state solutions possess asymptotic expansions for large and small Rossby numbers?

(*ii*) *Initial value problems based on the linearised equations*

Grace, Görtler Morgan and Stewartson have worked on such problems assuming the initial motion to be irrotational. The solutions are unique and in some cases the theoretical results agree with the experimental results. Squire (1956) has suggested an alternative scheme for linearization. This raises the following questions —

(*d*) What is the relation between the solutions of the linearised equations and the unsteady exact solutions?

(*e*) What is the spatial and temporal range of validity of the linear approximations and what is the correct procedure for obtaining higher order corrections?

(*f*) How are the steady and unsteady solutions related to each other?

(*g*) Can a satisfactory explanation of the discrepancy between the theory and experiment be given?

The above considerations prompted the author to undertake the investigations reported in the thesis.

N satisfactory answer has yet been given to question (a) although Lees has suggested an additional boundary condition to make the solution unique. The no-slip condition on the surface of the sphere suggested by Taylor leads to the paradox of zero wave-drag and is unnatural within the framework of perfect fluid theory. Answer to question (b) has been given by Nigam who showed that the non-linear equations of motion reduce to a single linear partial differential equation of second order. The solutions of this equation can be obtained in certain coordinate systems by the method of separation of variables.

The method of asymptotic expansions has been employed in the thesis to seek answers to questions (c), (d) and (e). An attempt has been made to provide a rational basis to the linear approximate theories. The perturbed flow at large Rossby numbers has been treated in detail but that for small Rossby numbers is still not completely understood and requires further investigation.

Developed by Lagerstrom and his associates for viscous flow at large and small Reynolds numbers.

The thesis is divided into five Chapters.

The first Chapter is a short review of the relevant experimental work done to-date on the subject of rotating fluids. The experiments of Taylor Long and Squire are briefly described.

The second Chapter deals with the exact solutions when the motion is steady For axisymmetric motion the non linear equations of motion reduce to one linear third order partial differential equation for the Stokes stream function Ψ The work of Taylor Long, Fadnis Fraenkel and Stewartson based on this equation, in connection with the uniform motion of symmetrical bodies along the axis of the rotating fluid is briefly described. The indeterminacy of the solutions due to the inadequacy of the usual boundary conditions of th inviscid theory is discussed Long's suggestion that a correct formulation of the additional field condition (not boundary condition) may lead to determinate exact solutions is also discussed. Two linear approximations to the equations of motion, which have been widely used in the study of motion in rotating fluids are described. The theoretical work of Grace, Görtler Morgan and Stewartson based on the linear equations is summarized. The discrepancies between the theory and the experiment are pointed out Finally the following two questions have been posed —

(1) What is the range of validity of linear approximations and what is the correct procedure for obtaining higher order approximations ?

(2) Do exact solutions of the steady-state problems possess asymptotic expansions for large and small Rossby numbers ?

In the third Chapter an attempt is made to seek answers to these questions The equations describing the motion of solids in incompressible inviscid rotating fluids have been expressed in non-dimensional form. Using the technique of Coordinate stretching the procedures for obtaining asymptotic expansions (for large and small Rossby numbers, Ro=U/ a, based on the characteristic length of the body) of the exact solutions in unbounded fluids are given and the spatial and temporal range of validity of these expansions is discussed The theory of asymptotic expansions is more or less satisfactory for large Rossby numbers, but for small Rossby numbers a number of difficulties crop up which have been discussed. Th asymptotic expansions of some known exact solutions are analysed and they are found to be in agreement with the theory developed in this Chapter

The conclusions arrived at are —

() For large Rossby numbers, the unsteady exact solution possesses inner' and outer expansions The outer expansion alone gives a solution which is uniformly valid to any order of approximation if a sufficiently large number of terms are retained. This makes the inner expansion unnecessary and this is due to the fact that the outer

and inner expansions are perfectly matched. An iterative method can be proposed for finding solutions for large Rossby numbers in unbounded fluids. The zeroth approximation satisfies the conditions at infinity. The first approximation satisfies a linear differential equation (same as given by Squire) and the equations for subsequent approximations can be easily obtained.

(ii) The small Rossby number case seems to be very mysterious. The unsteady exact solutions possess only one asymptotic expansion which becomes singular for large t in certain regions. The zeroth approximation satisfies a linear equation and the resulting flow is a rigid rotation of the entire mass of the fluid. The first approximation satisfies a linear equation and the solution is uniformly valid for any finite t, but singularities appear on certain surfaces when time approaches infinity. Higher order approximations can be obtained by solving non-homogeneous equations. In general the process is quite laborious.

(iii) The steady exact solutions possess asymptotic expansions of the required type for large Rossby numbers. The nature of the asymptotic expansions for small Rossby numbers is not known.

In the fourth Chapter the problem of slow uniform motion, after an impulsive start, of a sphere along the axis of rotation is considered. The solutions are given in ascending powers of t and are therefore valid for small values of t only. They bring out two experimental observations :—

(i) The fore and aft streamline pattern has no symmetry about the plane $x=0$.

(ii) The streamlines oscillate on the downstream side of the moving sphere.

Force along the axis of rotation is obtained and it is observed that it shoots up from zero to a finite value within a short time and after a few oscillations tends to a steady value. The expression for the force agrees with that given by Grace (1926). The solution confirms the conclusion reached in the third Chapter that the outer expansion alone gives a solution which is uniformly valid to any order of approximation if a sufficiently large number of terms are considered. The solutions satisfying the inner and outer conditions in unbounded fluids represent

(i) the outer solution correct to order R^{-3} for large Rossby numbers,

(ii) the inner solution correct to order unity for large Rossby numbers,

(iii) the solution for small Rossby numbers correct to order Ro.

In the end, the order analysis of the neglected terms is given and it is shown that the neglected terms are always of a lower order than the terms retained.

In the fifth Chapter procedure for obtaining asymptotic expansions (for large and small Rossby numbers) in bounded fluids (when the rotating fluid is enclosed in an infinitely long cylinder of radius b with its axis coinciding with the axis of rotation) are given Two cases arise —

(i) when the source of disturbance is infinitesimal

(ii) when the source of disturbance is a finite object

In the first case, the Rossby number is defined as Ro=U/Ωb In the second case it is not quite evident which Rossby number (U/aΩ based on the object or U/bΩ based on the radius of the cylinder) will enter into the description. The expansion procedures for the first case are developed in this Chapter Unlike the case of unbounded fluids, only one type of expansion exists for large Rossby numbers, while for small Rossby numbers the situation is similar to that in unbounded fluids and only one expansion exists The asymptotic expansions of exact solutions in bounded fluid for large Rossby numbers are obtained. The flow due to a sink (or source) placed at the axis of the rotating fluid for small Rossby numbers is found for small values of t. The ideas developed in this Chapter are confirmed by these examples

THE MECHANISM OF INGESTION PERISTALSIS AND EGESTION IN *PHERETIMA POSTHUMA* (L. VAILLANT) AND *EUTYPHOEUS WALTONI* MICHAELSEN

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The present investigation on the Mechanism of Ingestion Peristalsis and Egestion in *Pheretima posthuma* (L. Vaillant) and *Eutyphoeus waltoni* Michaelsen" consists of three parts—(I) Mechanism of Ingestion (II) Mechanism of the Movement of Food inside the Oesophagus and (III) Mechanism of Peristalsis and Egestion in the two earthworms. The more important facts found out in connection with each part are as follows —

PART I

OBSERVATIONAL

1 In *Pheretima posthuma* and *Eutyphoeus waltoni*, a part of the buccal chamber is everted during forward progression earth particles stick to its mucous layer and are carried inside along with it

2. *Pheretima posthuma* in addition to ingesting during locomotion, also feeds in the manner stated below

It becomes stationary and everts the wall of the buccal chamber in the form of dorsal and ventral lips which hold the food-particles between them and roll in After feeding a few times in this manner it invariably protrudes its pharyngeal bulb adpresses it against the substratum and then detaches it with a distinct jerk. Neither such a mode of feeding when the worm remains stationary nor the protrusion of the pharyngeal bulb have hitherto been observed in *Pheretima posthuma*.

ANATOMICAL

3 The buccal chamber in *Pheretima posthuma* and *Eutyphoeus waltoni* consists of two cavities—antero-buccal and the postero-buccal.

4 A system of parieto-enteric muscular strands is present in *Pheretima posthuma* and *Eutyphoeus waltoni* It consists of four set of muscular strands (parieto-buccal, parieto-pharyngeal, perieto-oesophageal and parieto-rectal) in the former and three sets of them (parieto-buccal, parieto-pharyngeal, and parieto-oesophageal) in the latter Such a system of muscular strands has not hitherto been described in the Oligochaeta.

5 In *Pheretima posthuma*, three septa—1/2, 2/3 and 3/4—are present

This is an abstract of the thesis submitted and approved for the Ph. D degree of the Agra University in the year 1960 The work for the thesis was completed under the kind guidance of Professor Be Charan Mahendra at the Department of Zoology Agra College, Agra.

in front of the septum 4/5 which was supposed to be the anteriormost (first by Bahl (1919) these evidently escaped his observation In *Eutyphoeus waltoni* there are no septa corresponding to the intersegments 1/2, 2/3, 3/4 and 4/5.

6 The septa in *Pheretima posthuma* and *Eutyphoeus waltoni* are divisible into four and three groups respectively on the basis of their attachment to the alimentary canal

7 The circular layer of muscle-fibres of the body-wall in *Pheretima posthuma* and *Eutyphoeus waltoni* is continuous with the circular layer of muscle-fibres of the buccal wall at the anterior end.

8 Muscle fibres of the parietal circular layer are compactly arranged in bundles which are imbedded in the matrix. In *Pheretima posthuma* the matrix is thick and the muscular bundles are arranged in two sets—a peripheral and an internal while in *Eutyphoeus waltoni* they are irregularly disposed, close to each other

9 Longitudinal layer of muscle-fibres of the body-wall in *Pheretima posthuma* and *Eutyphoeus waltoni* gradually diminishes forwards in the first few segments In these segments, its peripheral fibres diverge outwards and extend in the matrix of the overlying circular layer—a fact not hitherto described in the Oligochaeta.

10 The muscle fibres comprising the parieto-buccal muscular strands are continuous with the parietal longitudinal layer of muscle fibres parenchymatous connective tissue is present in relation with them and they are attached on the buccal wall in a manner not hitherto described in the Oligochaeta.

11 Bahl's (1936 1945 and 1950) description of the so-called lateral shelves of the pharyngeal wall in *Pheretima posthuma* is inaccurate They together form a single continuous structure which may be called a crescent-shaped shelf—a term in conformity with the facts and therefore more suitable. The pharyngeal wall in *Eutyphoeus waltoni* also has a crescent-shaped shelf.

12. The floor of the pharynx in *Pheretima posthuma* and *Eutyphoeus waltoni* is evaginated to form a median ventral diverticulum which has not been described before

13 The parieto-pharyngeal muscular strands are divided into two groups—an anterior and a posterior

FUNCTIONAL

14 In *Pheretima posthuma* the buccal wall, the parieto-buccal muscular strands, the buccal septa and the body-wall in the anterior segments partake in the process of eversion and retraction of the buccal wall.

15 The eversion and retraction of the buccal wall in *Pheretima posthuma* are accomplished in the manner described below

When the wave of circular contraction starts in the body-wall at the anterior end, it is also transmitted to the circular layer of muscle-fibres of the buccal

wall so that the latter elongates forwards. As this wave of contraction has passed on to the wall of the postero-buccal cavity the wall of the antero-buccal cavity is released of the circular pressure. Thus, in addition to elongating forwards, as it comes outside the mouth, it also dilates this dilation is perhaps enhanced due to unfolding of the longitudinal grooves. The so everted buccal wall is held outwardly swollen in a turgid condition by the longitudinal folds of the wall of antero-buccal cavity which have a thick covering of parenchymatous connective-tissue.

The retraction of the buccal wall is mainly done by the contraction of the parieto-buccal muscular strands. But simultaneously with their contraction the longitudinal layer of muscle-fibres of the buccal wall also contracts while the circular muscle-layer of the buccal wall and the muscle fibres of the buccal septa relax.

16. The buccal wall and the associated structures in *Eutyphoeus waltoni* are, on the whole, similar to those in *Pheretima posthuma* except for the absence of the buccal septa in the former

17 In the protrusion of the pharyngeal bulb in *Pheretima posthuma*, in addition to the pharyngeal bulb, the body-wall, the parieto-buccal, parieto-pharyngeal and parieto-oesophageal muscular strands, the buccal wall, the oesophagus and the oesophageal septa also participate.

18. The pharyngeal bulb is protruded and retracted in *Pheretima posthuma* in the manner described below

The parietal longitudinal layer of muscle fibres in the anterior segments and the parieto-buccal muscular strands synchronomously contract thereby dilating the mouth and pulling the buccal wall outwards so as to dilate the antero-buccal and the postero-buccal cavities. At the same time the non-glandular part of the oesophagus and the pharyngeal wall circularly contract so that the former elongates forwards and the crescent shaped shelf of the latter closes over the "conducting chamber As the oblique septal muscle fibres are in continuation with muscle-fibres of th circular layer of the oesophageal wall, they also contract and pull this part of the gut forwards so that the pharyngeal bulb protrudes.

The retraction of the pharyngeal bulb is done mainly by the contraction of the parieto-pharyngeal muscular strands.

Part II

Anatomical

19 The oesophagus in *Pheretima posthuma* and *Eutyphoeus waltoni* consists of a non-glandular part, a gizzard and a glandular part.

20 Glandular part of the oesophagus in *Pheretima posthuma* possesses calciferous glands which bear close similarity with those in *Pheretima hawayana* (Stephenson and Prashad, 1919)

21 Calciferous glands in *Eutyphoeus waltoni* are well developed and similar to those in *Eutyphoeus gigas* (Stephenson and Prashad, 1919)

22 Both the muscular layers—longitudinal and circular—in the wall of the non-glandular and the glandular parts of oesophagus in *Pheretima posthuma* and *Eutyphoeus waltoni* are well developed and have intimate relation with the muscle-fibres of the oesophageal septa—a fact which escaped the observation so far

23 Musculature of the gizzard in both the forms (*Pheretima posthuma* and *Eutyphoeus waltoni*) consists mainly of the circular muscle-fibres, although several radially and longitudinally disposed muscle fibres are also present.

24 The musculature of the oesophageal septa which had not hitherto been described in *Pheretima posthuma* and *Eutyphoeus waltoni* consists of two types of muscle fibres—radial and oblique.

25 Stephenson's (1930) generalisation that the muscle-fibres of a septum in Oligochaeta are mainly derived from the longitudinal muscular layer of the parieties is not true with regard to the septal muscle-fibres in *Pheretima posthuma* and *Eutyphoeus waltoni* In these forms, the septal muscle fibres merely pass through the longitudinal muscular layer of the body wall and extend beyond it into the matrix of the overlying circular layer

26 The radial muscle fibres of the septa start in the matrix of the circular layer of muscle fibres of the gut, while the oblique septal muscle-fibres are in continuation of the muscle fibres of this layer Fibres of both the types after running along the septum extend through the longitudinal muscular layer of the parieties and disappear in the matrix of the overlying circular layer

27 Two oesophageal septa—6/7 and 7/8—in *Pheretima posthuma* and three—8/9 9/10 and 10/11—in *Eutyphoeus waltoni* are conjointly attached to the oesophagus

28 While there are four sets of parieto-oesophageal muscular strands in *Pheretima posthuma* only one set of them is present in *Eutyphoeus waltoni*. They have not hitherto been described in the Oligochaeta

29 In *Pheretima posthuma* and *Eutyphoeus waltoni* the food inside the non-glandular and the glandular parts of the oesophagus moves backwards due to peristaltic movements of the oesophageal wall while it is checked from going in the forward direction due to the contraction of the oblique muscle fibres of the septa

30 The circular layer of muscle fibres of the gizzard is so developed that by its contraction not only the food which has already been crushed is pushed backwards into the glandular part of oesophagus but [illegible] triturated.

PART III

ANATOMICAL

31 The intestine in *Pheretima posthuma* and *Eutyphoeus waltoni* is divided into pre-typhlosolar typhlosolar and post typhlosolar (rectum) parts.

32. While in *Pheretima posthuma* the post-typhlosolar part of the intestine (rectum) considerably differs from the typhlosolar part in *Eutyphoeus waltoni* except for the absence of the typhlosole, there is no appreciable difference between them.

33 The rectal septa in *Pheretima posthuma* are thickly muscularised and the extent of their muscularisation progressively increases backwards thus they considerably differ from the intestinal septa. But in *Eutyphoeus waltoni*, they are not markedly different from the intestinal septa.

34 Parieto-rectal muscular strands are well developed in *Pheretima posthuma* but absent in *Eutyphoeus waltoni*

FUNCTIONAL

35 Anatomical facts indicate that in *Pheretima posthuma* and *Eutyphoeus waltoni* the food inside the intestine moves backwards mainly as a result of peristaltic movements of the intestinal wall

36. At the time of egestion in *Pheretima posthuma* and *Eutyphoeus waltoni* a strong wave of circular contraction sets in at the anterior end of the rectum (post-typhlosolar part of the intestine) and progresses backwards. As the oblique septal muscle-fibres are in continuation with the circular layer of muscle-fibres of the rectal wall they also strongly contract at the same time thus enhancing the effect of the circular contraction of the rectal wall

37 The voiding of pellets one by one in *Pheretima posthuma* and egestion of a continuous stream of the faecal matter in *Eutyphoeus waltoni* are evidently co-related with the anatomical differences in the rectum and the associated structures in the two forms

MORPHOLOGICAL STUDIES IN THE GRAMINEAE*

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The Gramineae is a unique family amongst the angiosperms. It has about 500 genera and 4000 species distributed all over the world. From the point of economic importance some of these head the list of useful plants and provide us with cereals, sugar scent, forage and many other articles of domestic needs.

From the stand point of floral morphology also the Gramineae is a characteristic assemblage of plants. The unit of inflorescence is a spikelet which is an aggregation of highly specialized florets. This family has attracted the attention of botanists and naturalists from very early time Schuster (1910) and Arber (1926-1931 1934) have made valuable contributions to our knowledge of floral morphology of the Gramineae. Recently Barnard (1957) has postulated a new hypothesis regarding the morphology of the spikelet and its various constituent parts. As regards embryological studies there has been little work on the Indian grasses considering the large number of genera and species and the great diversity of form exhibited by th family Most of the work that has been done on the embryology is confined to cereals and a few other grasses. More important f these works are those by Shadowsky (1926) on the antipodal apparatus in the Gramineae, Andersen (1927) on *Poa pratensis* and *P compressa*, Randolph (1936) on *Zea mays* Artschwager *et al* (1929) on sugarcane, Artschwager & McGuire (1949) on *Sorghum* and Narayanaswami (1952 1955) on the development of caryopsis in some Indian millets. Recently the phenomenon of apomixis has been reported in a number of grasses.

In the present investigation the structure and vascular anatomy of the spikelet of about 35 species belonging to eleven tribes of Gramineae has been studied in detail with a view to have a better understanding of the various floral organs and also the trends of specialization. Embryology and fruit development has also been studied for *Eleusine indica, Dactyloctenium aegyptium, Eragrostis poaeoides E. tenuloides E. coarctata* and *T agus biflorus*

Among the Festucoid (Pooid) grasses studied, members of the tribe Bambuseae, Hordeeae Arundineae and Eragrosteae have a multi-flowered spikelet while those of the Chlorideae, Zoiseae, Phalarideae, Stipeae and Thysanolaeneae have a single fertile flower in the spikelet. In the sub-family Panicoideae there is a two-flowered spikelet with the lower floret reduced to various extents and in extreme cases to its lemma only The upper or the second lemma generally bears a perfect flower

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The structure of the spikelet in the tribe Chlorideae where the florets above the first fertile floret are represented by their barren lemmas only suggests that the one flowered condition of thespikelet in the Pooideae has been derived from the multi flowered condition by suppression of the florets above the lemma I *Thysanolaena maxima* (Thysanolaeneae) shows an admixture of both festucoid and panicoid characters as regards its spikelet morphology There is present a barren lemma below the perfect flower and the rachilla also extends beyond the fertile floret and often bears a rudimentary second floret.

Increased number of glumes and the occasional continuation of the rachilla beyond the fertile floret as has been observed earlier by the author (Chandra 1958) in *Mnesithea laevis* a panicoid grass, certainly suggests that the one-flowered condition in the Panicoideae has also been derived through reduction from a multi flowered condition But in this case it is the second or the upper floret which is perfect and fertile It may be recalled that in the Pooideae it is the lower or the first floret which is perfect

Vascular supply of the spikelet has been found to differ in the two sub-families, Pooideae and the Panicoideae The fundamental difference lies in the origin of palea bundles which in the Pooideae arise directly from the vascular strand destined to supply the corresponding flower In the Panicoideae on the other hand they arise conjointly with the laterals of its lemma (Pa ea or the lateral bundles of the second empty glume lemma I lemma II and the two paleae arise conjointly as in some members of the Andropogoneae Such a condition in the Panicoideae where the bundles of the palea (which belongs to the flower axis) arise conjointly with the laterals of the lemma (which belongs to the rachilla or the spikelet axis) has been ascribed to extreme abbreviation of both the axes, so much so that their individuality becomes altogether obscure

The palea is bikeeled and two-nerved and is present on the adaxial side There are two cuniate lodicules which receive one bundle each. The bundle divides into a number of minute branches which ramify in each lodicule. Increased number of lodicules has been recorded for *Mnesithea laevis* and *Heteropogon contortus* both belonging to the tribe Andropogoneae As many as six lodicules have been observed in *M laevis* and three in *H contortus* The occasional presence of six and three lodicules suggests that the two-lodiculed condition generally met with in a majority of Graminea has been derived through reduction from the condition obtained in some of the bamboos where six l normally occur in a flower

Interesting variations have been recorded in the vascular supply of the gynoecium in *Dendrocalamus hamiltonii* Normally there is a placental bundle and three other bundles—one anterior and two laterals which ultimately pass out into the three stigmas In one instance there were six bundles The placental one anterior two antero-laterals and two postero-laterals The two antero-laterals, however vanished at various levels in the ovary

this case the placental bundle also continued upwards after supplying the solitary ovule In another instance there was only one extra antero-lateral bundle. In yet another case there were all the four bundles at the base but higher up only the anterior and the placental ones continued. In several gy noecia having otherwise normal vascular supply the placental bundle was found to continue after supplying the ovule. Gynoecia with increased number of vascular bundles have also been recorded by Arber (1926) From these observations it has been concluded that the grass gynoecium is composed of three carpels—one anterior and two laterals joined edge to edge The anterior bundle and the two postero-lateral bundles have been regarded as midribs or dorsals of these three carpels while the tv o antero-lateral bundles hich are occasionally present and the placental bundle as the fused ventrals of the adjacent carpels. The single ovule is born on the posterior side at the junction of the two lateral carpels and is supplied by the fused marginals of these adjacent lateral carpels. The placentation is obviously parietal The occasional continuation of the placental bundle upwards after supplying the single ovule gives a clue as to the vertical extent of the placentae in original f rms Attention has also been drawn to the trends of specialization in the gynoecium

The flower in the Gramineae has been interpreted as a branch arising in the axil of a bract—the lemma. The palea is equivalent to a bracteole or a prophyll. The lodicules are specialized structures representing the two ant ero-lateral perianth members of the inner whorl. The three stamens generally present, belong to the outer whorl the inner whorl being generally absent and present only in the tribes Bambuseae, Oryzeae etc The gynoecium has been interpreted as tricarpellary with one anterior and two postero-lateral carpels which are fused with their margins. The single ovule is borne parietally on the fused margins of the adjacent lateral carpels.

The three glumes present in the spikelet of *Paspalum compactum* have been interpreted as glum I glume II nd lemma II Lemma I with its subtended flower is interpreted to be lacking This is contrary to the conclusions of earlier workers like Blatter & McCann (1935) Bor (1940) etc.

The pedicelled spikelet of *Cymbopogon martini* has only one fertile male flower It has three glumes and th upper floret is generally taken to be bsent (Bor 1940) But it has been brought to light in th present in estigation that while the three glumes in this case re the first, second, and third (lemma I) respectively the flower present actually belongs to lemma II, that is absent, and not to lemma I. The orientation of the flower clearly bears out such an inference.

The present observations on the embryology of certain grasses are in full accord w th the data already recorded for other Gramineae The microsporogenesis is of the successive type and results in isobilateral tetrads of microspores. The pollen grain is 3-celled at the time of shedding and the vegetative nucleus is generally seen in a degenerated state in a mature pollen grain In *Eragrostis*

coarctata, however some abnormalities as for instance the formation of more than four microspores as a result of microsporogenesis, lagging chromosomes during the homotypic division etc. have been noticed. Some of the mature microspores in this grass are twice as big as others.

A single hypodermal archesporial cell differentiates in the nucellus and directly behaves as a megaspore mother cell that undergoes meiosis to form a linear or a 'T'-shaped tetrad of megaspores. Development of the embryo sac follows the *Polygonum*-type. The number of antipodals, however increases subsequently in some grasses.

Double fertilization *i.e.*, syngamy and triple fusion has been observed for *Eleusine indica*, *Dactyloctenium aegyptium* and *Eragrostis coarctata*.

The endosperm is of the nuclear type. Late in the development of the endosperm its surface layer behaves as a cambium cutting off cells towards the inner side and on the cessation of its activity becomes the aleurone layer of the mature caryopsis. In some cases the cambial activity of the surface layer is not very marked.

The development of the embryo follows the usual type met with in the grasses. In all cases studied a well defined epiblast is present. The seed-coat is formed by the two layers of the inner integument and the outer integument and the nucellus are completely absorbed. The pericarp is reduced to a very thin papery membrance in the form of a loose covering in *Eleusine* ... and *Dactyloctenium aegyptium* while in the three species of *Eragrostis* and *Tr*... *biflorus* it is adnate to the seed coat. The fruit in the former case is, therefore, an utricle and in the latter a caryopsis.

Certain ovule characters have been found to differ markedly in the two sub-families Pooideae and the Panicoideae. The outer integument in the Panicoideae is poorly developed and does not extend much beyond the style attachment region and on the micropylar side it is in the form of a hump of cells. In the Pooideae on the other hand it is quite well developed. In the Panicoideae nucellar epidermis below the micropyle divides to form a tissue which gives, though deceptively an appearance of a parietal tissue. In the Pooideae the nucellar epidermis does not divide. Another point of difference relates to the position of antipodals during the various stages of development. In the Pooideae the antipodals come to occupy a lateral position in the embryo sac prior to their degeneration while among the Panicoideae they continue to occupy their original chalazal position in the embryo sac until the time of their degeneration. It is suggested that these characters may prove to be of some importance in the systematics of Gramineae.

A STUDY OF VIRUS DISEASES OF SOME ORNAMENTAL AND OTHER PLANTS IN KUMAON*

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The study of a mosaic disease of garden petunia reveals that the disease is caused by a strain of cucumber mosaic virus. The disease is readily transmitted to different hosts by sap inoculation The virus has a wide host range and is able to produce infection in *Viola tricolor Passiflora edulis Beta vulgaris Spinacia oleracea, Dianthus sp., Cucumis sativus C melo Cucurbita pepo Zinnia elegans, Nicotiana tabacum* var Harrisons Special, *N tabacum* var White Burley *N glutinosa, N debneyi N rustica* and *N glauca, Nicandra physaloides Lycopersicum esculentum, Solanum nodiflorum, S melongena, Capsicum annuum C frutescens Datura stramonium* and *Petunia hybrida. Nicotiana tabacum* var Havana and *N langsdorffii* found to be symptomless carrier of the disease

The physical properties of the virus resemble those of cucumber mosaic virus. Its dilution end point is 1 10 000 thermals inactivation point is 65 C-70°C, and the longevity in vitro is 72 hours when sap is kept in an incubator (20° C)

The disease is transmitted by *Myzus persicae Brevicoryne brassicae, Aphis gossypii* but not by *Macrosiphum pisi*. *Myzus persicae* was proved to be most efficient vector in transmitting the disease. The behaviour of the virus is of non persistent type as the virus is acquired by the insects in a short period of two minutes and the aphides lose their infectivity soon after feeding on test plants.

Aphid *Myzus persicae* can carry the virus infection to more than one plant if ten aphides are serially transferred to the successive plant. The infectivity is lost by one hour fasting after infection feeding period.

The infectivity of the virus is increased ten times by the use of an abrasive.

The virus gets inactivated when treated with (*a*) Alcohol 10% (*b*) Copper sulphate 1% (*c*) Formaline 1% (*d*) Nitric acid 2% (*e*) Silver nitrate 2% (*f*) Sodium hydroxide 0.2%

Cross Protection and Serological tests have confirmed that the *Petunia* mosaic disease under present study is caused by a strain of cucumber mosaic virus.

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Nasturtium Ringspot Virus

Aphis gossypii has been proved to be a new vector in the transmission of Nasturtium Ringspot Virus. Other vectors like *Brevicoryne brassicae* and *Myzus persicae* can also transmit the disease but are less efficient than *Aphis gossypii*. Plants on which viruliferous *Aphis gossypii* have fed show symptoms earlier than those on which other two aphid vector fed. *Macrosiphum pisi* failed to transmit the disease. The vector virus relationship showed that Nasturtium Ringspot Virus is of non persistent type.

Plum Line Pattern Virus

Line pattern disease of plums occurs in the orchards of Chaubattia, Shyamkhet, Ramgarh and several other localities where plum is grown. The disease was found occurring naturally on different plum varieties i.e., Green gage, Japanese plum, Chaubattia Maynard, Ramgarh Maynard and Sharp's Early. The percentage of infection in orchards ranges from 35% to 80%.

The symptoms of plum line pattern virus are confined to the foliage and are extremely variable, consist of light green, yellow or creamy white patterns on leaves. The patterns most commonly encountered include Vein banding, Oak leaf and Chlorotic spotting.

These symptoms are more pronounced on leaves which appear early in spring as yellow vein chlorosis and become creamy white and rusty brown in late autumn.

The disease is readily transmitted both by *grafting or budding* of diseased scion wood to healthy Chaubattia Mynard, Green gage, Ramgarh Maynard and Sharp's Early.

In addition to plum varieties the disease is transmitted to certain peach varieties, peach seedling, Moor Park apricot and also apricot seedlings. Peach seedling is a good indicator of plum line pattern virus.

It has been found that the disease is not fully systemic in all the growing parts of the infected trees.

Several attempts to transmit the virus to herbaceous host gave negative results. Infected leaves give positive colour tests for the presence of virus.

Apple Mosaic Virus

Apple mosaic virus has been found in different orchards of Kumaon in several commonly grown apple varieties. The incidence of the disease ranges from 20% to 40% in the apple growing areas so far visited. The common symptoms observed are recognised in four pattern viz. (a) White or pale yellow flecks; (b) Large white or pale yellow areas extending over the large inter venal areas; (c) Vein banding where primary, secondary, sometimes tertiary veins are bordered by white or yellow strips; (d) Necrotic areas which are usually found in later

chlorotic areas. These patterns occurred only on leaves produced in spring and summer and persisted till the leaf fall.

The disease has been transmitted by budding or by grafting diseased scion wood to different healthy apple varieties.

Apple mosaic virus is present in two strains in Red Astrachan apple variety The presence of these two strains have been confirmed in varieties Delicious, Jonathan and Rymer

It has been found that the disease is not fully systemic in all growing twigs of the infected trees and healthy plants have been propogated out of such infected plants.

The disease could not be transmitted to any herbaceous hosts.

The incidence of infection decreased when cuttings or seedlings were treated in hot water at 37° C for ten minutes daily for four consecutive days. Higher temperatures kill the host tissue.

Colour tests suggested for the presence of stone fruit viruses gave positive results in infected leaves.

MORPHOLOGICAL AND ANATOMICAL INVESTIGATIONS ON ARTOCARPUS FORST

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Artocarpus Forst. (Artos-bread, Carpos-fruit) is a unique tropical genus with about 50 species distributed from Indian Archipelago to China They are trees of 50-150 feet height and have evergreen or deciduous stipulate leaves The genus belongs to the sub-family Artocarpoideae which is included in Urticaceae or in the family Moraceae by different authors.

The genus has attracted attention from very remote past because of its economic importance. Some of its species yield best types of wood and well known fruits like, Jack fruit (*A heterophyllus*) 'bread fruit (*A altilis*) Chempedak' (*A. integer*) and 'monkey jack (*A lakoocha* and *A rigidus*) Besides, the genus has considerable taxonomic palaeobotanical, anatomical, morphological commercial and biochemical interest.

Perhaps the most important part of the plant is its inflorescence and this has been given rather meagre attention by morphologists and anatomists and for that reason our understanding of it is not clear The way the individual flowers are borne and the part they play in the formation of composite fruit require a detailed investigation Another feature which is worth investigation is the occurrence of phenomenon of cauliflory in *A heterophyllus* This phenomenon as a whole has received very little attention and in *Artocarpus* it has been almost ignored so far

In the present investigation I have been able to work out eight species of *Artocarpus* collected and procured from different localities in India as well as Malaya. They are *A heterophyllus* Lamk., *A integer* (Thunb) Merrill, *A altilis* (Park.) Fosb., *A lakoocha* Roxb., *A. rigidus* Blume, *A hirsutus* Lamk., *A. odoratissimus* Blanco and *A. chaplasha* Roxb *A. heterophyllus* has been studied in greater detail and cauliflory also has been worked out to some extent in this species.

The study of internode shows that it is of uniform type in all species and has same structural plan The development of thick cuticle, dense growth of woolly hairs and early origin of cork cambium in *A. rigidus A chaplasha*, *A. odoratissimus* and *A lakoocha*, etc., suggest that these plants have developed xerophytic characters. Their variability in different species are important morphological and taxonomic features

The presence of laticifers is a regular feature in all the species. They are more common near the vascular tissue and phloem regions of different

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organs but their frequency varies from species to species. The crystals of various kinds particularly druses are common in the cortex and phloem regions of vegetative parts of all the species.

There is considerable uniformity in the nodal anatomy of the various species. All are multilacunar but there is some variation in the number of traces departing from the main stele. They get arranged in an oval ring but seven or more traces departing earlier supply the leaf while the rest of the traces enter the two stipules.

There is a normal simple ring of vascular bundles in the petiole. In cases where the leaves are larger in size there is found an additional medullary ring with in the normal one as in *A. chaplasha*, *A. lakoocha* and *A. altilis* etc.

The stipules are quite characteristic in all the species. They enclose the future leaves, stipules and buds, etc. and leave a prominent characteristic annular scar on the node. In *A. lakoocha* however the stipules are slightly different. They enclose the buds for a short period and become free and later [illegible] soon after. On the basis of vascular supply the stipules here are considered as an outgrowth of leaf base.

The morphology of inflorescence reveals two peculiarities (i) Occurrence of the so called polystelic condition in the stalk and the inflorescence axis [illegible] the down turning in the course of the vascular supply of the lower flowers. The former condition is understandable if we consider every one of the [illegible] steles as representing a branch. Thus it is inferred that the apparently [illegible] spike of *Artocarpus* is probably the outcome of earlier fusion or coalescence of several fertile branches. Several examples have been cited in favour of this inference. In the light of the information available the following two views regarding the evolution of this type of inflorescence have been tentatively suggested.

Firstly this type of inflorescence might have been evolved from [illegible] forms with loose clusters or panicle type. After condensation of branches [illegible] due to intercalary growth in the basal region the vascular supply of lower flowers get pushed upward possibly resulting in *Artocarpus* type of inflorescence.

Second possibility is that by downward growth of the margin of a flat or convex type of coalesced receptacle (*Dorstenia brasiliensis* type) and [illegible] intercalary growth in its central region the present day condition of [illegible] and its downturning supply of lower flowers is obtained. A correct [illegible] ed answer however can be given after anatomical investigations of other [illegible] simpler forms.

The male flowers in *Artocarpus* appear to be much reduced both in [illegible] and non-essential parts. The two segmented condition of perianth [illegible] have been derived from a four segmented one. The [illegible] stamen [illegible] is perhaps on the [illegible] of further reduction to staminodal condition [illegible] to sterility of the flower.

There are present small scaly and pointed structures intermixed with the male flowers. Their anatomical and vascular supply evidently suggests that they are the reduced perianth of sterile flowers. The nail like structures present in *A. rigidus*, *A. hirsutus* and *A. lakoocha* etc., have a single strand in the stalk which goes up to the peltate head. These have been tentatively interpreted as interfloral bracts.

The female flower shows both modifications and reductions in their non essential and essential whorls. The perianth of female flowers is tubular and gamophyllous and appears to consist of two segments. Like that of male flower it is free from each other in young condition. Before anthesis however it starts fusing in the middle region dividing the perianth into three parts: (i) the lower free region (ii) the middle fusion region and (iii) the upper free region. In *A. lakoocha*, however, the third region is not visible.

The gynaeceum in majority of the flowers is reduced and abortive. It is bicarpellary and has become pseudomonomerous in some and monomerous in others, due to partial or complete suppression of second carpel. In many such cases no trace of its second dorsal is visible. However in some cases the stylar and stigmatic parts of the suppressed carpel may still persist.

There is some anatomical evidence which indicates that there might have been more than two carpels in the ancestral forms of *Artocarpus*. In *A. altilis* the central plexus left after the supply of usual two dorsals expands on two sides and gives off incipient traces to one or two extra carpels.

The ancestral carpels had more than one ovule in its single locule. Such an inference is borne out by the placental supply of extra ovule in *A. heterophyllus*. The placentation is regarded as an axile. In cases where the second carpel is completely suppressed, the fertile region of carpel extends over towards the suppressed carpel.

After anthesis, the whole female inflorescence develops into a multiple or composite fruit. This is a spurious type of fruit and the actual achenial fruits are insignificant and remain embedded. The so called fruit of *Artocarpus* is highly specialized and consists of three parts: (i) fruit axis (ii) persistent perianth and (iii) small achenial fruits. Of these perianth is the most important and significant unit, which has undergone considerable modifications and specialization. It shows the usual three parts of perianth as found in flower condition.

In *A. heterophyllus* the lower free region is edible and grows vigorously particularly in fertilized flowers. In *A. altilis* the fusion region extends to the basal region and forms the edible part. In *A. lakoocha* however there is no outer free region forming the rind and both lower and upper regions are edible. The quality of the fruit depends upon the amount of its edible region.

Cauliflory has been worked out in *A heterophyllus* and some minor field observations on the subject have been recorded. It has been observed for instance that buds developed on normal terminal shoots fail to produce inflorescences during flowering season. But those developed on older branches in basipetal fashion, are dormant buds and may produce small sized inflorescences. Generally these are unable to develop further and rarely produce small sized fruits. Buds developing on still older limbs and trunk of the tree appear to be dormant ones to begin wtih which develop exogenously and produce flowering shoots that develop into normal inflorescences and fruits. Soon after these areas become cork clad and raised up and in the next flowering season there starts the *de novo* protrusion of secondary buds at these sites. These buds are interpreted here as *endogenous* buds because they originate from deeper layers of the cortex.

It is concluded that in *Artocarpus heterophyllus* there is an advanced type of cauliflory where normal flowering and fruiting is completely shifted to the limbs and main trunk of the plant

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A STUDY OF VIRUS DISEASES OF ECONOMIC PLANTS OF U P (INDIA)

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Cucurbits, chillies, tomatoes and several cruciferous plants a e commonly grown as vegetable crops throughout Uttar Pradesh In most places they have been seen infected w th different viruses The present work embodies investigations on different aspects of some of the viruses found infecting these crops.

A mosaic disease of radish (*Raphanus sativus* L.) due to a distinct strain of cabbage black ringspot virus has been described. The present strain slightly differs from the type virus in ts storage time and in its reaction with some host plants. It produces only local lesions on cabbage, cauliflower and Brussels sprout, and symptomless local infection on *Nicotiana glutinosa* L. and *N. rustica* L. It also causes symptomless systemic infection in *Petunia hybrida* Vilm. The symptoms of the disease are pronounced during summer (28°C) and diffused in winter (below 15°C)

The other crucifer plants seen naturally infected by this virus include turnip, *Brassica juncea* var *rugosa*, stock, candytuft and sweet rocket. *Lepidium ruderale* L., a common weed, is recorded as a harbouring host of the virus. Seed beds of stock, candytuft and sweet rocket act as a source of infection to the crops

The virus produces countable local lesions on tobacco So the quantitative studies were made on the properties of the virus The dilution end point of the virus differs according to the variety of the test plant used. Quantitative experiments show that there is a considerable drop in infectivity at a dilution of 1 100 and that there is only a negligible drop in infectivity at a dilution of 1 10 Carborundum powder when used in the inoculum increases the dilution end point by 100 times. The thermal inactivation experiment shows that there is a large temperature co-efficient for the virus In the critical range the increase in the rate of inactivation with relatively small increase in temperature, is quite great. The infectivity of the virus is also reduced by centrifugation. The extracts from spinach, *Phytolacca acinosa* *Capsicum frutescens* L. and *Datura stramonium* L. inhibit the infectivity of the virus.

Both *Aphis gossypii* Glov and *Myzus persicae* Sulz. can transmit the virus. A detailed study of the vector virus relationship was made with *A. gossypii* The efficiency is much increased if aphids are first starved and then fed for only a few minutes on infected plants. Aphid soon cease to be infective but single aphids can cause more than one infection

This is an abstract of the thesis submitted and approved for the Ph D Degree of the Agra University for th year 1960

The virus is not transmitted through the seeds of infected plants of [illegible] and candytuft.

A study of mosaic disease of vegetable marrow (*Cucurbita pepo* L.) has been made. Affected plants show severe distortion malformation and extreme reduction of lamina. These plants become very weak and bear few fruits, which are distorted and are small in size having a rough surface.

The causal organism of the disease has been identified as a strain of [illegible] melon mosaic virus. In addition to vegetable marrow the other cucurbits [illegible] found naturally infected by this virus include *Cyclanthera pedata* Schrad, *Cucurbita maxima* Duche and *Cucumis sativus* L. Susceptibility of different cucurbits to this virus varies according to the species. Of the various plants tested vegetable marrow is found to be the most susceptible while the susceptibility of *Cucumis sativus* is very low.

The host range of the virus is limited to Cucurbitaceae only. Its thermal inactivation point is between 55-60° C dilution end point 1 10,000 and longevity *in vitro* 8-10 days at 20°-22°C.

The virus can be transmitted by *Aphis gossypii* and *Myzus persicae*. Detailed study of vector-virus relationship with *A. gossypii* shows it to be of a persistent nature.

The virus is also transmitted through seeds of *Cucurbita pepo*.

A vein banding mosaic disease of chillies (*Capsicum frutescens* L.) due to tobacco veinal necrosis virus is mechanically transmitted to different commercial varieties of chillies and also to other solanaceous plants. *Myzus persicae* and *A. gossypii* can transmit the disease after short infection-feeding. It is not transmitted through seeds of chillies. The virus resembles potato virus Y in its physical properties and host range but differs from it in its reaction towards tobacco in which it induces necrosis of veins, petiole and stem. Serologically it is related to potato virus Y but both do not cross-protect in tobacco plant. It is, therefore regarded as a variant strain of potato virus Y similar to tobacco veinal necrosis virus.

Tomato (*Lycopersicon esculentum* Mill.) plants have been seen infected [illegible] potato virus X. Affected plants show mosaic mottling and necrosis of the [illegible] It resembles the type strain in its physical properties and also in the host [illegible] Countable local lesions are produced on inoculated leaves of *Gomphrena* [illegible] L. and *Chenopodium album* L. The sap from infected plant gives positive [illegible] with antiserum against potato virus X and negative reaction with [illegible] against potato virus Y tobacco mosaic virus and cucumber mosaic virus.

SOIL CONDITIONS IN RELATION TO SOME DISEASES OF CROPS

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Part One of the thesis comprises of investigations on root-rot of *guar* (*Cyamopsis psoraliodes* DC.) and wilt of gram (*Cicer arietinum* Linn.) both caused by *Sclerotium rolfsii* Sacc. These are soil-borne diseases and quite prevalent in Uttar Pradesh (India) The fungus attacks the two hosts both before and after emergence of seedlings. In the pre-emergence infection, the seeds are enveloped in a fungus mat of mycelium and lose the power of germination. In post-emergence seedling infection the cortical tissue at the collar region usually decays and the plants droop down and finally wilt away In the case of *guar* with the advance of disease symptoms in the shoot the root system also starts rotting Partial wilting, a characteristic phenomenon of vascular wilts, was also observed in gram.

As soil conditions profoundly affect such diseases, pot culture experiments were conducted to study the effect of soil temperature, moisture, reaction, organic content (humus) and texture on the root-rot and wilt. The combined influence of some of these factors was also investigated. All the data obtained on total percentage mortality (pre and post-emergence mortality together) were analysed statistically following the simple method of analysis of variance

Influence of soil temperature on the disease development was conducted in soil temperature tanks designed after Wisconsin temperature tanks maintaining the different temperatures viz. 20° 25° 30° 34° 38° and 40°C in aluminium pots. Sensitive toluene thermostats (± 20°C) were used to regulate the required temperature ranges. It was found that 25-30°C is the optimum range for root-rot development in *guar* (72 %) and 30°C fo maximum wilt development in gram (84%) These observations directly correspond with the growth of the parasite (*Sclerotium rolfsii*) in culture its maximum linear growth being 105.3 mms. at 30°C.

In a study of the effect of soil moisture, four levels viz 10 15 20 and 25% on oven dry weight of the soil which are well within its water holding capacity were employed. It was observed that the incidence of both the diseases is greater in low soil moisture. In th case of *guar* the maximum total mortality (76.67%) was recorded in 15 % soil moisture while in gram the maximum (93.33%) was in 10%. The minimum disease incidence was observed at 25% soil moisture in both the crops, the figures being 29.33% in *guar* and 26.66% in gram.

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The pH effect was studied in sand cultures and a modified Shive mineral solution was supplied to each pot at regular intervals in equal quantity. The suitability of the nutrient solution was previously established for both the crops. Six pH levels viz., 4 6, 5 6, 6 6, 7 4 8.4 and 9.2 were maintained by adding N-solution of hydrochloric acid and sodium hydroxide to the nutrient solution in various proportions. The maximum disease incidence both in rot of *guar* and wilt of gram occurred in the acidic range while alkaline conditions retarded the same. In *guar* the highest total mortality was obtained in pH 6 and in gram at pH 5 6 the mortality figures being 54 16 and 89.58 , respectively. The minimum disease expression was recorded in pH 9.2 in both *guar* and gram, the values being 6.24 and 20 83 , respectively

Organic matter (humus) of the soil was altered by adding manure compost to the soil in different proportions Five such treatments were experimented with and the data indicate that the effect of organic content (humus) more pronounced in lowering mortality if the percentage of humus is appreciably high In normal garden soil, the incidence of the disease was high, the figures being 71 88 in *guar* and 82 14% in gram while in compost alone only 31 25 and 35 7% respectively

Five types of soils viz., clay clayloam loam sandyloam and sand having varying textures in relation to the diseases of *guar* and gram were employed and it was noticed that the maximum disease incidence was in loam (medium light). It decreases considerably in light and heavy soils.

Two types of soil (manured and unmanured) distinctly acidic and alkaline and with two levels of soil moisture (low and high) were considered for the combined effect of these factors. It was observed that the combination of low soil moisture with unmanured soll and acidic reaction gave the highest mortality both in *guar* and gram, the actual values were 97 5 and 90 0 respectively When a combination of high soil moisture with manured soil and alkaline reaction was employed the total mortality was minimum, 2 ... and 30 in gram

Part Two of the thesis deals with the very important and widely prevalent stem-gall disease of coriander (*Coriandrum sativum* Linn) caused by *Protomyces macrosporus* Ung This is also a soil-borne disease and causes galls on stems, leaves and fruits Affected fruits show hypertrophy and ... The effect of soil reaction on the disease development has been studied ... Since chlamydospores are the primary cause of infection their germination has also been studied in relation to pH to find out correspondence if any with incidence of the disease The various data are analysed statistically

It was observed that the host plants are susceptible at all the pH ... (pH 4 6 5 6 6 6 7 4 8 4 and 9 2) maximum percentage of ... occuring in pH 7 4 and decreasing on either side more so in the acidic than the alkaline ranges Maximum infection of fruits (seed) occurs at pH ...

8 4 the % of diseased fruits being 43 9-40 1 acidic as well as highly alkaline conditions tend to reduce the disease. The distribution of the disease relatively on stem pedicel and fruits is nearly the same in a given pH level except in pH 7 4 and 8·4 where the proportion of intensity is approximately in the ratio of 1 1 : 2 indicating high fruit infection at these two pH levels. The total disease intensity increases progressively (4 62 to 33 40 /) with rise in pH from 4 6 to 7 4-8 4 But further increase in pH lowers it.

The germination of chlamydospores is normal at pH 6 6-8 4 reaching a percentage of 78 6 at 7 4 As mentioned above the disease is favoured by alkaline soil reaction (pH 7 4-8 4) and thus a close relationship is indicated between total disease intensity and germination of chlamydospores.

The remaining part of the thesis consists of two additional papers.

MORPHOLGICAL AND ANATOMICAL STUDIES IN POLYGALA CEAE AND ITS ALLIED FAMILIES

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The present work emodies the result of investigation in 32 species belonging to the families Polygalaceae and Vochysiaceae The genera *Xanthophyllum* and *Diclidanthera* of the controversial monotypic families Xanthophyllaceae and Diclidantheraceae respectively have been studied under Polygalacea .

The inflorescence in *Xanthophyllum* as studied in X *affine* is a compound one where units of three flowers each are arranged in a racemose fashion on the main inflorescence axis. On the basis of external morphology and vascular anatomy each unit has been interpreted as a dichasium whose peduncle has condensed so much that every flower of the dichasium appears to arise directly from the main axis.

In other members of Polygalaceae where inflorescence was studied it is found to be a simple raceme. On the basis of comparative morphology it has been concluded that the original condition in Polygalaceae is a branched racemose inflorescence and it is through the condensation of the lateral axes that we get the prevalent simple raceme.

A detailed study of the vasculature of the inflorescence axis, in order to determine the origin and disposition of floral traces, was made in two species of *Polygala*—*P vulgaris* and *P arillata* In both species—as also in other species of Polygala—the angle of divergence between two successive flowers is 140° The entire vascular supply of the inflorescence axis was found to be arranged in distinct pattern along a set of independent lines, similarly the floral traces from these lines also follow a set sequence in their origin and disposition. In *P vulgaris* the number of lines along which vascular supply is arranged, is five and every successive sixth flower on the axis receives its floral trace from a common line. In *P arillata*, though, in the base of the inflorescence the number of lines is five it is reduced to three only towards the tip With this change in number of lines, there is also a change in sequence from successive sixth flowers to successive fourth flowers which receive their floral traces from a common line.

A detailed account of the external morphology of the flower in the tribe Polygalae has been given because of the paucity of information in the literature. Lysigenous pouches were observed in the sepals as well as in the 'keel of *Polygala eriaptera*.

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The controversial placentation of the tribe Polygalae is interpreted as axile both topographically and anatomically The carpel cords on the septal radii are interpreted as compound bundles formed by the close association of the inverted placental strands and the lateral strands The earlier suggestion that they are ventral bundles is held to be incorrect. The septum is suggested to be formed by the fusion of the true carpellary margins and not by the fusion of the placental outgrowths as suggested earlier Evidence for this has been obtained from comparative morphology and also from the orientation course and behaviour of the vascular bundles supplying the placentae and the ovules

In *Securidaca* and *Monnina*, the gynoecium appears to be monocarpellary The anatomical evidence in case of *Securidaca* clearly indicates its pseudomonomerous condition. In *Monnina marginata* however the dorsal supply is completely missing and hence it is difficult to interpret it as a pseudomonomerous condition There is no vascular supply in the style of *Monnina marginata*

The flower of the tribe Polygalae is suggested to have been derived from an original hexamerous one. Evidence for it has been found in the display of twelve radii in the origin and disposition of the traces to the peripheral organs. The keel is interpreted to be a compound structure formed by the structural synthesis of a median sepal and two adjacent petals. The earlier view that the keel is a petal, is discredited both morphologically and anatomically

The eight stamen condition of the flower of tribe polygalae is again concluded to have been derived from original twelve stamens, of which four stamens from the radii of the three outer sepal and the keel have been lost in the present day forms.

In the tribes Moutabae and Xanthophyllae, pentamery appears to be an established fact.

On the basis of the present morphological studies it has been concluded that *Polygala arillata* and *P triphylla* are so distinct from the genus *Polygala* that they should be raised to the ranks of different genera within the tribe Polygalae A close examination of the material of *P triphylla* proper and *P triphylla* var *glaucescens* has revealed that the characters exhibited by the latter are of far greater importance than simply varietal. On this basis it has been suggested that both these varieties should be given the status of separate species in the proposed new genus.

A discussion about the status and relationships of the genus *Xanthophyllum* is made and it is concluded that though *Xanthophyllum* represents a thoroughly natural group readily recognisable by a number of strong characters it appears to represent a divergent line from a stock common to the members of the tribe Moutabae. Relationships and position of the genus *Diclidanthera* within the tribe Moutabae have also been discussed in the same discussion

A detailed study ofthe stamens with special reference to the structure and mode of dehiscence of the anthers in the family Polygalaceae was made and it has been concluded that the apical poricidal dehiscence prevalent in the anthers of the tribe Polygalae is derived from a typical longitudinally dehiscing anther as exhibited by *Xanthophyllum*. The anthers in *Xanthophyllum* are somewhat dorsifixed while in the rest of the genera of Polygalaceae, they are basifixed. Excepting the genus *Polygala* all the genera studied exhibited four sporangiate anthers. *Polygala* however possesses in addition to four-sporangiate anthers, three-sporangiate and bisporangaite anthers as well. *Polygala arillata* and *P triphylla* var *glaucescence* and *P myrtifolia* possess four-sporangiate anthers, while three-sporangiate anthers were found in *P crotolarioides*. From a comparative study it has been concluded that bilocular condition prevalent in majority of the species of *Polygala* is the result of the suppression of the two ventral pollen sacs.

The anther of the tribe Moutabae dehisces by a tangential slit, while that of *Xanthophyllum* by two longitudinal slits one in each anther lobe In the tribe Polygalae, *Polygala arillata* and *P triphylla* var *glaucescence* are the only ones where the anthers dehisce by typical tangential slits. In the other members, however the dehiscence is either by a single ventral slit or by a slit which represents somewhat a transition between a tangential slit and a ventral slit.

The structure of the style and stigma varies from species to species but in almost all the species of *Polygala* studied for their pollination mechanism, there is some sort of a pocket at the distal end of the style in which the anthers release their pollen. It is shown that style and stigma are instrumental in making the escape of pollen outside the keel which encloses the essential organs. Though the entire mechanism of the flowers appears to be adopted for cross pollination, what was observed in majority of the species of *Polygala* points towards the regular occurrence of autogamy Pollen grains from the same flower were observed to germinate on the stigmas.

Pollen grains in the family Polygalaceae were examined and it was found that the pollen exhibited by *Polygala triphylla* is different from those of the other members.

A prominent intrastaminal disc was observed in *P triphylla*, a fact which does not seem to have been reported in the species so far The disc in *Polygala arillata* *P triphylla* var *glaucescence* and *Xanthophyllum* is vascularised by phloem tissue derived from stamen traces Stomata were also observed on the disc of *P arillata*.

The placentation in the tribe Moutabae is axile, while in tribe Xanthophyllae it is parietal The parietal placentae of *Xanthophyllum* are traversed by inverted placental strands. Thus has been considered as an important lead for the suggestion that the parietal placentae have been derived from axile placentae.

ON THE STRUCTURE, DISTRIBUTION AND INNERVATION OF THE SPECIAL HEART MUSCLE SYSTEM OF MAMMALS*

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One of the outstanding controversies in cardiac physiology which still remains unresolved is that of the myogenic versus the neurogenic theory of cardiac conduction. The neurogenic theory was favoured in earlier years when almost all investigators believed that nerves and ganglia were present in the heart of vertebrates for the initiation and conduction of the cardiac stimulus of contraction. Supporters of the myogenic theory showed the presence of specialized muscle systems formed of sinuatrial node, atrioventricular node, atrioventricular bundle and Purkinje fibres in the heart of vertebrates to initiate, control and conduct the contraction stimulus. A few investigators located the presence of nerves and ganglia in the nodes and the bundle in favour of the neurogenic theory of cardiac conduction. Some of the recent investigators have denied the presence of any specialized muscle system in the form of nodes and bundles in the heart of mammals. According to them it is the nervous component of the heart which is capable of impulse initiation and conduction. The conclusions of many investigators are based on the results obtained by them by studying the heart of one or two types of mammals. In the present thesis, therefore, the heart of various mammals has been examined to present the evidence that the specialized tissue exists and is accompanied by nerves to help in the initiation and conduction of the heart beat in mammals.

A difference of opinion also exists with regard to the phylogeny of the impulse conducting system of mammals. The early investigators of eighteenth century believed that the nodal and Purkinje fibres constituting the impulse initiating and conducting tissue of mammals is remnant of more extensive tissues of similar nature found in the heart of poikilothermal vertebrates. Against these observations the anatomists of twentieth century have held the view that the impulse conducting tissue in the heart f mammals is neomorphic in nature. The third view which has been very lately advanced maintains that the mammalian cardiac conducting system is neither remnant of that of lower vertebrates nor neomorphic in nature but has evolved from and is a further specialization of the tissue already existing for the purpose in the heart of lower vertebrates. In the present investigation the heart of various mammals particularly of marsupials has been studied to determine th phylogeny of the specialized cardiac conducting tissue of mammals.

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The thesis includes seven papers dealing with the specialized muscle system and its nervous component of the heart in representative types of the class mammalia The important and new facts discovered in each type are –

The heart of 116 m. m. embryos of oposum has been studied with particular reference to its conducting system A well defined and distinct sinuatrial node has been observed at the sinuatrial opening An atrioventricular node and an atrioventricular bundle with its two limbs are also present. The two nodes and the bundle are composed of 'Purkinje fibres However the Purkinje fibres were not observed in any other portion of the heart. The muscle fibres of the interatrial septum connect the sinuatrial node to the atrioventricular node which is continued caudally and ventrally into the atrioventricular bundle The right and the left limbs, in which the bundle of His bifurcates caudally descend down over the respective sides of the ventricular septum. A continuous muscular pathway has been traced and described for the conduction of the stimulus of contraction from sinus venosus to ventricles. The multiple muscular connections of Kent are not present and the bundle of His has been observed to be the only connecting tissue to convey the atrial stimulus of contraction to the ventricles.

The structure and distribution of the specialized tissue forming the conducting system in the heart of the shrew has been described. A sinuatrial node an atrioventricular node, and an atrioventricular bundle with its two branches have been located and described. All these structures are formed of Purkinje fibres Purkinje fibres are also observed in the walls of atria and in the interatrial septum. These fibres are, however not present in the ventricles. The horse-shoe shaped sinuatrial node is connected with the atrioventricular node and the atria by the Purkinje fibres The oval atrioventricular node is in free communication cranially with the muscle fibres of the interatrial septum and caudally with those of the atrioventricular bundle. An atrioventricular bundle has been observed to connect the atria with the ventricles. It divides into a right and a left branch which run along the respective sides of the ventricular septum. No multiple muscular connections of Kent could be observed. It is presumed that the cardiac contraction impulse which would originate in the sinuatrial node would be conveyed to the atrioventricular node through the Purkinje fibres present in the interatrial septum. The atrioventricular node will pass these impulse after necessary delay to the atrioventricular bundle which would distribute them through its right and left branches to the respective sides of the ventricle The myogenic theory of impulse initiation and conduction has been supported.

In the heart of bats the impulse initiating and conducting tissue is formed of sinuatrial node, atrioventricular node, atrioventricular bundle Purkinje fibres ordinary cardiac muscle fibres and multiple muscular connections of Kent. The heart of bats possesses multiple muscular connections of Kent in addition to the bundle of His for a quick transmission of the cardiac stimulus

of contraction from atria to ventricles. In this feature the heart of bat resembles that of birds and differs from that of mammals. A continuous muscular pathway exists to convey the cardiac stimulus of contraction from one chamber of the heart to the other The cardiac impulse initiating and conducting tissue of birds and mammals develops in accordance w th the functional requirements of the heart of individual animals The nervous component of the specialized muscle system as seen in the heart of bat has been described. Nerves and ganglia were observed in the atrial walls and in the vicinity of sinuatrial and atrioventricular nodes.

Sinuatrial node atrioventricular node, atrioventricular bundle are present in the heart of rabbit as important constituents of the impulse conducting pathway Purkinje fibres are present in the walls of the two atria and in the interatrial septum of the rabbit s heart. The bundle of His is the sole connecting tissue between the atria and the ventricles of the heart. Multiple atrioventricular connexions or Paladino-Kent bundles are absent. Nerves and ganglia could not be located either in the sinuatrial and atrioventricular nodes or in the bundle of His.

With the disappearance of the sinus venosus in the heart of the guinea pig, a well defined and distinct sinuatrial node composed of Purkinje fibres' is present to originate the cardiac stimulus of contraction. The atrioventricular node which is present in the caudal portion of the interatrial septum is very much reduced and poorly differentiated. An atrioventricular bundle, which is the only tissue to connect the atria with the ventricles, is present at th cephalic end of the interatrial septum. Sinuatrial node, atrioventricular node, atrioventricular bundle and the ordinary muscle system connecting the two nodes and the bundle all form a continuum to explain and affirm th presence of the muscular conduction system in mammals. Nerves and ganglia were not observed to accompany th nodes or the bundle

Specialized impulse initiating and conducting tissue formed of S. A. Node, A. V Node and A. V Bundle is present in the heart of squirrel. Sinuatrial and atrioventricular nodes are connected through specialized as well as ordinary muscle fibres present in the interatrial septum. Purkinje fibres have been shown to be important and integral constituents of impulse conducting system. Nerve elements in relation to th specialized muscle system of the squirrel s heart are described.

The structure and distribution of th specialized conducting tissue of the heart of armadillo has been studied in serial sections. A well developed kidney shaped sinuatrial node is present in the crista terminalis at the cranial end of the interatrial septum. The sinus venosus is absent. The opening of superior venacava into the right atrium is guarded by two valves Th sinuatral node is connected with the atrioventricular node through the muscular interatrial septum. A poorly differentiated atrioventricular node is situated

in the caudal portion of the interatrial septum. The fibres of A. V Node communicate freely with those of the A. V Bundle and the interatrial septum. The atrioventricular bundle, which is a well developed structure, lies at the caudal extremity of the ventricular septum. It divides into a right and a left branch which extend downwards for a short distance over the respective sides of the ventricular septum. The atrioventricular bundle is connected with the A. V Node by Purkinje fibres The A. V Bundle is the only connecting tissue between the atria and the ventricles. Multiple muscular connexions of Kent are absent. S A. Node, A. V Node and A. V Bundle are all formed of Purkinje fibres Thus the pathway for the transmission of cardiac contraction stimulus is formed of both the ordinary myocardium and the Purkinje fibres

BIOLOGY OF SOME VEGETABLE PESTS AND ANATOMY OF PRE-IMAGINAL STAGES

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Bagrada picta Fabr

1 From 58 to 150 eggs were laid per female in 6 days.
2. On the 4th day the colour of the white spherical egg changed to pink, then brown by the 9th day after which it hatched.

3 The 1st stage nymph did not resemble the adult in size colour and in being wingless.
4 Interval between each moult was from 4 to 5 days.
5 In the 5th stage nymph, striking changes occurred —

(i) Prominent wing pads developed.
(ii) Rostrum so far 3 segmented became 4 segmented.
(iii) Sexes were differentiated.

6. After the 5th moult the adults emerged having the following characters —

(i) Tarsi instead of having 2 segments became 3 segmented.
(ii) Antenna which were 4 segmented became 5 segmented.
(iii) They were fully winged

7 Life-history was completed in 30 to 32 days.
8. The final instar nymph of *Bagrada picta* Fabr is almost globular flat and shaded deep-brown with distinct head thorax and abdomen

9 The conical head has most of its sutures obliterated and posterior most portion of the head is telescoped within the pro-thorax.

10 The spherical compound eyes are prominent, the rostrum is fully developed as in the adult and the antenna is also well developed but only four segmented.

11 Pro and meso-thorax are clearly visible but the meta-thorax is indistinct. The last two thoracic segments have well developed wing pads of which the anterior pair covers the posterior pair

12. Each of the thoracic segments have a well developed pair of thoracic legs the pro and the meso-thorax have a pair of spiracles each

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13 The abdomen is flat and tapering posteriorly with the first two segments longest and the widest, and the segmentation marked with deep grooves.

14 First six abdominal segments have a pair of spiracles on the outer lateral margin of the sternite which is rather indistinct.

15 There is a marked sex differentiation in this instar the males being smaller darker in colour and the terminal abdominal segments also differ in structure.

16 The study of the internal organs of the final instar nymph of *Bagrada picta* Fabr shows that all organs have developed completely as in the adults except that of the reproductive system.

17 Digestive system may conveniently be divided into fore mid and hind-gut and the mid-gut is distinguished in three regions as in some other Heteroptera.

18 Only salivary glands are present as transparent bodies on either side of pharynx with the accessory organs and delicate ducts.

19 There are two thoracic and six abdominal spiracles. The head receives two branches and the two posterior segments few branches from the respective terminations of the lateral longitudinal trunks on either side that join the spiracles Each segment receives the ventral commissure, visceral branch and the dorsal and lateral branches from the lateral longitudinal trunk near the spiracles.

20 Excretory system consists of four pairs of convoluted, coiled, delicate yellowish malpighian tubules that end blindly and buddled up in the posterior portion of the body cavity

21 The nervous system shows a high degree of concentration All the thoracic ganglia are fused in a single mass to give three pair of nerves to the thorax and all the abdominal ganglia have again fused in a single mass and give a pair of branch to each segment.

22 Sexes are separate, females have four pinkish ovarioles, common oviduct an accessory gland and a blind duct while the males have only the testes and a blind duct.

Epilachna chrysomelina Zimm

1 On an average a female lays from 31 to 52 eggs in 7 days.

2. The delicate barrel-shaped, white egg turn deep yellow on the 4th day increase in size and become brown on the 7th day

3 The incubation period is about 8 days.

4 The newly hatched larva is the scarabaeoid type with mandibulate mouth-parts, 3 distinct thoracic legs, 10-segmented body with branched scoli on the dorsal surface.

5 The 1st, 2nd, 3rd and 4th instar larvae have 8, 10 12 and 16 branches in the scoli.

6. Duration of each instar is four to five days and the different stage larvae are similar in shape and colour

7 The number of branches in the scoli and the size of the body increase in the successive instars.

8. *Pupa is immovable and curved or C-shaped.*

9 Life history is completed on an average in 44 days during March-April

10 The final instar larva of *Epilachna chrysomelina* Zimm. is cylinderical, well built, tapering posteriorly with distinct head, thorax and abdomen.

11 Prognathous sub-oval, chitinized, yellowish head, bent downwards with prominent antenna, ocelli and inverted V-shaped white marking in front.

12. Sutures and sclerites mostly obliterated.

13 Mouth-parts consist of a pair of dark chitinized mendibles a pair of maxilla with three segmented palp and the labium and hypopharynx on the lower or ventral side.

14 Pro, meso and meta thorax clearly demarcated and the pro-thorax bearing a pair of spiracles on its lateral aspects.

15 On each segment six branched scolli present in a median transverse line dorsally and a pair of well developed thoracic legs along with few setae.

16 Ten segmented abdomen tapering posteriorly with clear segmentation. Posterior-most segment forms the posterior adhesive disc to help the larva in locomotion

17 Dorsally six branched scoli present on each segment parallel to that of the thorax. The posterior scoli being shorter and less branched

18. First eight abdominal segments have a pair of spiracles on the dorso-lateral aspect

19 Anatomical study of the final instar larva of *Epilachna chrysomelina* Zimm. reveals that all the systems have reached almost complete development as the adult except the reproductive system.

20 The digestive tract may easily be divided into fore, mid, and hind-gut of which the first and the last mentioned are comparatively simpler and the mid-gut is coiled and convoluted and of variable diameter.

21 Respiration is carried on with the help of nine pair of spiracles (one thoracic and eight abdominal) lateral longitudinal trunks and the segmental branches.

22 Only mandibular gland is present as delicate structures on either side of the fore-gut opening separately at the base of the mandibles.

23 Malpighian tubules are six in number delicate, long, coiled and convoluted, forming the cryptonephric system and finally opening in the ileum.

24 Nervous system confirms the typical plan except that the abdominal ganglia show a tendency of condensation as they are approximated to each other and restricted upto the fifth abdominal segment. Each ganglion sends off a pair of delicate branch to the respective segments.

Acherontia styx West.

1 The female lays 57 to 88 eggs within three to five days.

2 The light milky spherical eggs become green and oval on hatching after ten days.

3 The first-stage larva comes out of the egg on the eleventh day

4 The larva is a typical lepidopteran 'Polypod larva with segmented body indistinct head, thorax and abdomen bearing the characteristic recurved horn

5 There are five larval instars.

6 During moulting the form remains the same but the colour and size changes.

7 For pupation the last instar larva goes about seven inches below the surface of the soil

8 The pupa is an obtact pupa, with legs, wing cases, antennae eyes, and abdominal segments being marked distinctly

9 It completes its life-cycle in about 46 days.

10 Long robust, cylinderical, leathery beautifully coloured, distinctly segmented and annulated final instar larva with distinct head, thorax and abdomen

11 Trianguar hypognathous, dark coloured head with prominent inverted Y-shaped clypeal marking and well developed antenna ocelli and mouth-parts.

12. Gnathal appendages consists of sub-rectangular flat labrum and a highly chitinized toothed pair of mandibles dorsally (forming the upper lip) and a fused structure, maxillo-labial-hypopharyngeal complex on the lower side (forming the lower lip) composed of labium, hypopharynx and the maxillae.

13 Three thoracic segments clearly marked and almost black. The pro-thorax bears a spiracle laterally and each segment has the well developed thoracic legs ventrally

14 Ten segmented robust cylinderical abdomen with eight pair of spiracles on the first eight abdominal segments six pairs of pro-legs on the third, fourth, fifth, sixth and tenth abdominal segments. The eighth segment has the horn on the mid-dorsal portion and the anal plate attached to the dorsal posterior margin of the tergite

15 Each pro-leg supplied with a number of minute sharp curved chrochets that help in locomotion, the last pair being the best developed.

16. A study of the setae on the body of the larva show a degree of variation from its previous instar

17 The food canal is almost a straight tube of varying diameter which can easily be divided into fore, mid and hind-gut Mid-gut is longest, thickest and the most prominent portion of the digestive system.

18 Both salivary and silk glands are well developed. The former are small delicate white bodies attached on either side of the fore-gut opening at the base of the mandibles separately while the latter are long tubular coiled, thick yellowish structures reaching upto the seventh abdominal segment and coils along with the malpighian tubules.

19 The first thoracic and first eight abdominal segments bear a pair of well developed spiracle each provided with the closing mechanism. Head has the typical dorsal, ventral and the median head trunk while the posterior most segment receives only a number of tracheoles. Rest of the segments have four branches each, dorsal, ventral, visceral branches and the ventral commissure.

20 Excretion is effected by six long delicate, yellowish malpighian tubules and an excretory chamber no cryptonephric system is present in this case but the malpighian tubules end blindly

21 Nervous system is typically developed with sub-oesophageal ganglia, three thoracic and eight abdominal ganglia, a pair of delicate branch given in each segment from the ventral ganglion.

19 Anatomical study of the final instar larva of *Epilachna* [illegible] Zimm. reveals that all the systems have reached almost complete development as the adult except the reproductive system.

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5 There are five larval instars

6 During moulting the form remains the same but the colour and size changes.

7 For pupation the last instar larva goes about seven inches below the surface of the soil.

8 The pupa is an obtect pupa, with legs, wing cases, antennae eyes, and abdominal segments being marked distinctly

9 It completes its life-cycle in about 46 days.

10 Long, robust, cylinderical, leathery beautifully coloured, distinctly segmented and annulated final instar larva with distinct head, thorax and abdomen.

represented as a pair of undifferentiated gonads supplied with a blind duct.

20 The digestive tract is extremely simple and consists of a straight tube of varing thickness. Fore, mid and hind-gut can be differentiated. The mid-gut is longest, prominent, white muscular and occupies a major portion of the body cavity

21 Salivary glands are represented as delicate transparent bodies on either side of the pharynx, while the silk glands are tapering thick tubes on either side of the mid-gut reaching up to the six abdominal segment from the base of the spinneret.

22. Respiratory system consists of one thoracic and eight abdominal spiracles, two longitudinal tracheal trunks and tracheoles.

23 Six malpighian tubules along with th excretory chamber comprise the excretory system. A conspicuous reunion of the malpighian tubules with the rectum give rise to cryptonephric system

24 The nervous system is typically developed on the general plan with supra sub, oesophageal ganglia, three thoracic and eight abdominal ganglia Each segment receives a pair of nerves from the respective ganglion.

22 Gonads are premature and only a gonad body with a blind duct has been recorded

Pieris brassicae Linn

1 The female lays forty five to seventy-four eggs in 5 days.

2 The pear-shaped creamy egg becomes yellowish and elongated on hatching after about six days.

3 The first stage larva comes out of the egg on the seventh day

4 The larva is a polypod caterpillar with indistinctly segmented body.

5 There are five larval instars, each with five to seven days duration.

6 In each moult the form of the body remains the same but the colour and size changes.

7 The final instar larva is well developed, blackish in colour gregarious in habit and voracious feeder

8 For pupation either it goes under the soil or attaches itself to the leaf surface.

9 The pupa is brown immovable, non-feeding with wing-case appendages, and segments indistinctly marked.

10 Period of pupation is the same in both the cases being eleven days.

11 The life history is completed in about fifty-five days.

12. The final instar larva of *Pieris brassicae* Linn. is soft cylindrical, dark coloured, fleshy with a distinctly segmented and annulated body which can be demarcated into head, thorax and abdomen clearly

13 The oval hypognathous head is almost black with few setae and prominent antenna, ocelli and mouth parts.

14 The gnathal appendages consists of the labrum mandibles and the maxillo-labial-hypopharyngeal complex.

15 Pro-thorax with few setae, a pair of spiracles, the thoracic shield and a pair of pro-legs, meso and meta-thorax are identical with only a pair of thoracic legs each

16 Abdomen ten segmented, first eight segments having a pair of spiracles each on the lateral sides, third, fourth, fifth, sixth and tenth segments having a pair of pro-legs each and the eighth abdominal segment having the anal shield.

17 Pro-legs help in locomotion and are provided with short, curved numerous crochets.

18 The chaetotaxy of this instar is strikingly different from that of the previous instars.

19 The internal organs of the final instar larva of *Pieris brassicae* Linn. are well developed except that of the reproductive system which is

RESPIRATORY ADAPTATIONS IN SOME OF THE SILUROID FISHES OF INDIA

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It has generally been observed that the fishes belonging to the family Siluridae are able to survive longer out of water than the carps. Late Dr. S. L. Hora, Director, Zoological Survey of India, suggested this problem to be tackled as follows —

1. List the Siluroid fishes commonly available and note in detail their ecology, how and where they live.

2. Find out for each the period of survival out of water by carrying out experiments and grade them according to their capacity to live out of water.

3. Seek through dissections and sections of the lining of the buccal cavity and gills as to which can be respiratory in order to enable these fishes to live out of water for a longer time. The structure of the skin is also to be studied. About *Rita* Day states that it can live out of water for 40 minutes. So a beginning can be made with this fish.

The Siluroid fishes that have been taken into account are —

1. *Rita rita* (Hamilton)
2. *Wallago attu* (Bl. & Schn.)
3. *Mystus cavasius* (Hamilton)
4. *Heteropneustes fossilis* (Bloch)
5. *Clarias batrachus* (Linnaeus)
6. *Ompok bimaculatus* (Bloch)
7. *Eutropiichthys vacha* (Hamilton)

These fishes have naked skin and do not possess any accessory respiratory organ except *Heteropneustes fossilis* and *Clarias batrachus*. Morphological and histological studies of the various regions like buccal cavity, gill, gill cavity, alimentary tract and skin of these fishes were undertaken to find out if any of these is adapted for accessory respiration to enable the fish to sustain its life out of water for a long duration of time. It was observed histologically that skin in these fishes may to a large extent be capable of conducting respiratory exchange of gases. The skin in the case of *Rita* presents a rough rugged surface while in the other fishes it is quite smooth, slimy and very thin permitting easy exchange of gases from and to the blood. A study of the skin of these fishes has

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been undertaken both morphologically and histologically and experiments have been conducted to correlate their efficiency as accessory respiratory organs.

Various authors have dealt with the respiration of fishes. The skin in the case of eel, *Anguilla anguilla* has been studied by Krogh (1904) and others, who have described its skin to be respiratory with a capillary system similar to that of frog but with the capillaries less densely crowded. Haddon (1889) has described caudal respiration in *Periophthalmus*. Aestivation by the fishes by burrowing in the mud has been studied in the case of *Lepidosiren*, *Protopterus*, *Symbranchus*, *Pseudapocryptes* and *Amia*.

No work has been done on the skin of Siluroids acting as an accessory respiratory organ. A critical study of the skin revealed its function as an accessory respiratory organ.

Live specimens of fish were taken for experimental and histological studies. Skins for histological studies were taken from live specimens and fixed in Bouin's fluid or 10% to 15 % formalin.

Sections 6 microns to 10 microns thick were cut and stained with Delafield's haematoxylin and counterstained with eosin.

External features and ecological notes on the seven fishes enlisted have been described. *Rita rita* is a stockily built fish, capable of living out of water for a pretty long time and can be conveyed fresh for long distances. It is valued as food by the poorer classes, but is a very foul feeder. The fish is carnivorous, feeding on fishes and dead bodies. It seeks shelter especially in winter under rocks and stones at the bottom. The histology of the skin of the fish indicated two layers, the epidermis and the dermis. The epidermis consists of club cells, layer of ordinary epidermal cells and mucous cells. The dermis or the corium consists of connective tissue fibres. It contains blood vessels. The upper portion of the corium is raised into papillae like projections into the epidermis. Most of these papillae reach almost to the uppermost region of the epidermal layer. Through these papillae the blood capillaries and pigments also enter the epidermal region of the skin. Thus the blood capillaries reach the upper region of the epidermis separated from the outside atmosphere by a few layers of epidermal cells. The epidermis and dermis are separated by a thin noncellular basement membrane. *Wallago attu* is a very powerful voracious and foul feeder and hence it is popularly known as Freshwater Shark. Because of its voracious habits it is not popular for stocking ponds. Its flesh is not well favoured but on account of its cheapness and the absence of intermuscular bones it is esteemed as food. Histologically the epidermal region of the skin is stratified, 50 to 65 microns thick. It consists of the usual cells—club cells, ordinary epidermal cells and mucous cells. Nuclei pigment and blood capillaries lie below the basement membrane. The dermis does not form any papillae like projections into the epidermis.

Eutropiichthys vacha—Its form of body nearly resembles to that of Indian Trout'. Unlike *Rita* and *Wallago* it is an active fish. It is a clean feeder confining itself mostly to the column region of the water. It is a delicate fish. It probably likes running water. The skin consists of the usual two layers—the epidermis and dermis. They are also of the usual type. The epidermis is about 28 microns to 50 microns thick. The basement membrane is very thin.

Heteropneustes fossilis is a dark purplish brown to almost brown black fish with an accessory posterior air sac extending backwards on either side of the neural spines amongst the muscles above the abdominal and part of the caudal region originating from the gill cavity. It communicates with the mouth by a small slit, which serves as a passage for air. The fish is esteemed as food. The fish is omnivorous. It is a bottom dweller preferring muddy soil and since it possesses accessory respiratory organ it can survive in that environment and even out of water for considerable time. The epidermis of the skin consists of the usual cells and is about 71 microns to 92 microns thick. A distinct basement membrane is present.

Clarias batrachus is a greatly elongated fish with a long spineless dorsal fin which is characteristic. The fish possesses an accessory branchial apparatus in the form of two beautiful, coral like dendritic air trees. It is lodged in a recess above and behind the gill cavity. It lives in shallow muddy ponds marshes and sluggish rivers. It is able to breathe atmospheric air direct. Its diet consists of insect larvae, shrimps, worms and algae along with other vegetable debris. The epidermal region of the skin is about 85 microns to 92 microns thick. A continuous layer of pigments is present below the basement membrane.

Ompok bimaculatus is silvery glossed with gold coloured with a dark oval shoulder spot situated just above the middle of the pectoral fin on the lateral line. It is good eating. This fish prefers the column region of water. The skin consists of the usual two layers—the epidermis and dermis. The epidermis is 45 microns to 60 microns thick. A basement membrane is present but is not clearly distinguishable.

Mystus cavasius is greyish above and yellowish beneath. A black spot is always present on the head end of the lateral line. It is a small fish growing not more than 6 inches in length. The epidermis of the skin is 65 microns to 71 microns thick and consists of the usual component of cells. Basement membrane is very thin and almost imperceptible. Pigment is present below the basement membrane and also between the layers of connective tissue fibres.

In order to test whether any respiratory exchange of gases takes place through the skin, the following simple experiments were conducted. The experiments were conducted at different times of the year.

1 The survival time of fishes out of water under dry and moist conditions was noted. In dry condition, the fish were kept bare on the earth under the shade. In moist conditions, the fishes were surrounded with aquatic weeds with occasional sprinkling of water

2 Survival time of fishes under both dry and moist conditions by enclosing the head region including the opercular openings in a piece of rubber balloon sheath and thus eliminating head region to conduct any respiratory exchange was noted.

3 Survival of fishes out of water was also noted when the head region is enclosed in a rubber balloon sheath and the remaining skin portion also smeared with dry talcum powder

4 The fish with enclosed head were kept in an air tight flask having a piece of glass tubing fixed through the rubber stopper and inverted over a strong solution of KOH. The rise of KOH in the glass tubing was noted which was due to the evolution of Co_2 from the skin into the flask.

5 Lethal temperature of the fishes in the higher reaches of temperature was noted for each fish

The comparison of the observations of experiment no 1 and 2 indicate that the fish died earlier in experiment no 2 and the fishes lived longer in moist condition than in the dry condition

In experiment no.3 the fishes died much earlier since all the respiratory surfaces were blocked to perform any respiratory function.

In experiment no 4 there was a rise of KOH in the delivery tube indicating evolution of Co_2 from the skin surface.

Comparison of experiment nos 1 2 and 5 indicate that under same conditions the carps in experiment no 5 which are scaly fishes lived for a shorter time.

Experiment no. 6 was conducted to see the condition of fishes at the lethal temperature when they overturned in warm water and began to show signs of death When the purely water breathing fishes at this stage are transferred to water of normal temperature they survive and begin to swim normally after an interval of a few minutes. While on the other hand air breathing fishes have to be subjected to direct atmosphere first and then transferred to fresh water of normal temperature This experiment indicated that except for *Heteropneustes* and *Clarias* all these fishes are non-air breathing depending on aquatic respiration alone

As the lower strata of water are deficient in oxygen content, the fishes living in that environment have to develop extra respiratory organs to supplement the normal branchial respiration in order to utilise the maximum amount of oxygen available in the medium. The fishes taken for study here have been found to have their skin functioning as a Supplementary respiratory organ in addition to their normal breathing organs. This conclusion is supported by the histological study of the skin It is noticed that the skin in these fishes is

very thin and identical to that of frog through which the frog is capable of performing cutaneous respiration. A comparison of the thickness of the skin in these fishes have been made and compared to that of frog

It has also been observed that the bases of the fins become red due to the great supply of blood in that region in a foul aquatic medium. A cross section of the skin of this region shows a profuse and great accumulation of blood in the blood capillaries underneath the epidermis. The epidermal layer in this region is very thin and it is, therefore, quite possible that respiratory exchange of gases takes place in this region. The thinness of the skin permits an easy diffusion of oxygen which is not possible in the scaled skin of the carps

The skin in these fishes are primarily meant for aquatic respiration in oxygen deficient medium. In the case when a fish leaves water the skin also utilises atmospheric oxygen to maintain the vital processes of existence for sometime.

It has been observed that a greater surface area of the skin of *Rita* with dermal papillae is equally efficient as the accessory respiratory organs of *Heteropneustes* and *Clarias*

The investigation made here is of some economic importance to the fish marketing in India. The fishes can first be anaesthetized by putting a ball of cotton wool saturated with alcohol into its mouth The fish thus anaesthetized can then be wrapped in wet cloth or aquatic weeds and packed in wooden boxes. Thus the fishes can be transported in live condition during the journey of a few hours. This will reduce the freight charges normally incurred in transporting fish in live condition in water tanks. During the journey occasional sprinkling of water may be carried out.

STUDIES ON SOME AMPHISTOMATOUS TREMATODES OF DOMESTICATED ANIMALS

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The purpose of the present investigation was to determine the life-history and taxonomic position of a few species of amphistomatous trematodes, with particular reference to amphistomes of veterinary importance. A survey of the cercarial infections in different species of aquatic snails was carried out in Bareilly from 1955 to 1958. It was observed that amphistome cercariae were very common. The adults and the taxonomic position of some of these cercaria were unknown. Studies were undertaken to determine their morphology life-history and taxonomy.

1. During the survey a rich collection of more than 9000 live specimens of common aquatic snails found in local rivers, ponds and pools was made during different seasons.

2. Snails belonging to the genera *Lymnaea*, *Melanoides*, *Bithynia*, *Indoplanorbis*, *Gyraulus*, *Vivipara* and *Pila* were examined for cercarial infections.

3. The specimens of snails were seperated according to species and examined individually for trematode infections.

4. A record of the occurrence of other types of cercariae was also maintained.

5. Only those amphistome cercariae morphology and/or life-history of which were unknown were selected for further investigations.

6. It has been found that the snails *Gyraulus convexiusculus*, *Indoplanorbis exustus*, *Bithynia pulchella*, and *Lymnaea luteola* act as natural intermediate hosts of different species of amphistomes in Bareilly.

7. The morphology of the amphistome cercariae was studied from materials obtained from both naturally and artificially infected snails.

8. Three amphistome cercariae have been proved experimentally to develop into *Gigantocotyle explanatum* (Creplin, 1847) Näsmark (1937), *Ceylonocotyle scoliocoelium* (Fischoeder 1904) Näsmark (1937) and *Cotylophoron indicum* Stiles and Goldberger (1910) respectively.

9. The morphological features of the various developmental stages in the life cycle of the above mentioned amphistomes, together with that of the adult stages, have been described and discussed in detail.

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miracidium have been studied fully The rate of development, at regular in tervals, of the miracidium has also been studied

The miracidia of this fluke hatch in 11 12 days in summer months and 18-20 days in winter months. The miracidia hatched out in 9-13 days when the eggs were kept at 32 35°C in an incubator The process, of hatching and penetration of the miracidium and general activity have also been studied and included in the thesis. The active miracidium penetrates into the snail *Bulinus pulchella* which serves as an intermediate host under experimental as well as natural conditions. The sporocysts were recovered from the experi mentally infected snails 11 days after infection in the month of October The sporocyst is an elongated body and contains developing rediae and germ balls. The rediae come out from the sporocyst in an immature condition and attain maturity in the snail tissue. The redia gives rise to immature cercaria which emerges from the redia into the snail tissue and finally attains maturity there. The morphology of the sporocyst and redia and the rate of development of the redia have been described in detail. The biology of the intermediate snail host, *Bulinus pulchella* experimental infection of the snails in the laboratory and the reaction of the snails to the miracidium have been studied in detail The development of the cercaria inside the snail tissue has been followed. The morphology of the cercaria, its shedding time and the activity are studied in detail.

The mature cercaria first emerges from the snails 31 days after the infection and is characterised by the presence of well developed oesophageal bulb, dif fuse body pigment, the main excretory canals with an arch shaped transverse excretory connection which forms loop on e ther side of the body w th the main canal. The median excretory diverticulum is absent. The cercaria of *C scoliocoelium* belongs to Pigmentata group of Sewell (1922) After a free swimming existance the cercaria encysts on grass blades. The process of encystment and the morphology of the metacercaria have been tudied in detail Clean goats were fed encysted cercariae and their faeces became positive for the eggs of the parasite 173 days after infection. Subsequently the adults were recovered from their rumen and identified as *Cylicocotyl scoliocoelium* (Fischoeder 1904) Nasmark (1937) The detailed description of the adult parasite is aslo included.

13 The history of the genus *Cylicocotyle* and the systematic position of the species *C scoliocoelium* have been given in the thesis. A key for the identification of various species of *Cylicocotyle* has also been furnished.

14. The third amphistome cercaria collected from *Indoplanorbis exustus* when fed to a clean goat developed into *Cotylophoron indicum* Stiles and Goldberger (1910) The cercaria has been identified as *Cercariae indicae* XXVI Sewell (1922) The original description of this cercaria has been supplemented with additional accounts of their genital system The different redial stages ha e been described fully

15 The history of the genus *Cotylophoron* and the systematic position of the species *C indicum* have been discussed in detail.

16 A table containing the characters on the basis of which *C. indicum* and *C cotylophorum* were seperated by Stiles and Goldberger (1910) has also been included in the thesis

17 Morphology of the two new amphistome cercariae, their rediae and metacercariae have been studied in detail. One of the cercaria belongs to Pigmentata and the other to Diplocotylea group

18 Both the cercariae were found to parasitise the snail *Indoplanorbis exustus*

19 *Cercaria bhalerai* n sp is characterised by black pigment in the form of small dots distributed all over the dorsal surface of the body the eyes are in the form of large dark black patches of pigments, definite eye lenses are absent, cross-excretory connection together with the median diverticulum form a 'T' shaped structure and the excretory granules are arranged in groups leaving a space between them

There are two generations of redia in this cercaria. The first generation of redia contains one to three small second generation of rediae together with germ balls. The second generation contains developing cercariae and germ balls. The long gut of the redia is lined by large cells *The cells are provided* with distinct nucleus and fill the cavity of the gut. The morphology of the cercaria, redia and the metacercaria have been described fully

20 The new cercaria resembles closely *Cercaria indoplanorbis* Peter and Srivastava (1955) but can be seperated from it on the basis of body pigmentation, position of the rudiments of the ovary and Mehlis s gland, shape of the cross excretory canal, size of the median excretory diverticulum position of the vitelline glands, presence of large cells in the gut of the redia and the presence of the second generation of redia.

21 *Cercaria mathuraensis* n. sp is characterised by the presence of black pigment only on the anterior half of the body and mostly between the two caeca, the excretory canals are much coiled and form loops at places, a large portion of the posterior part and a little portion of the anterior part of the main excretory canals are devoid of excretory granules, the redia is provided with two pairs of locomotor appendages and the gut of the redia is very big and is filled with dark black and brown pigments The morphology of the cercaria, redia and the metacercaria have been studied fully

22 The only cercaria which comes very close to this new cercaria is *Cercaria barailly* Peter and Srivastava (1955) But the two can easily be seperated by the arrangement of the body pigmentation, coiling of the main excretory canals absence of excretory granules in the major part of the posterior portion and a little part of the anterior portion of the main excretory canals,

length of the oral pouches and the presence of dark brown pigment in the gut of the redia

23. The systematic positions of both the cercariae have been determined

24 A list of different species of amphistome cercariae described so far from India is included in the thesis

25 The occurrence in goats and sheep of *Olveria indica* Thapar and Sinha (1945) and *O bosi* Tandon (1951) have been reported for the first time

26 A report of the species of molluscs examined for trematode infection localities and sources from which they were obtained and the types of cercarial infection found in them have also been included in the thesis

27 Seasonal variation in cercarial infection in molluscs, with particular reference to the larval infection of *Gigantocotyle explanatum*, *Ceylonocotyle scoliocoelium* and *Cotylophoron indicum* during different months of the year has been studied

28. The effect of cercarial infection in snails has also been studied.

29 A few cases of double infection in snails have been recorded.

30. A description of some amphistome infected areas near about Bareilly has also been included in the thesis

The classification, life histories and economic importance of amphistomes have also been discussed.

RADIOACTIVE DECAY CHARACTERISTICS OF THREE ODD A ISOTOPES OF OSMIUM, GADOLINIUM 159 AND COBALT 55

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Disintegration schemes of a few odd A isotopes have been studied with the help of a beta-ray spectrometer (thin lens type) and scintillation spectrometers. NaI (Tl) and anthracene crystals were used to detect gamma rays and beta rays respectively Two types of coincidence circuits have been used to study the coincident relationship between gamma rays or gamma rays and beta rays. One type consisted of a slow coincidence circuit with resolving time $J\sim 0.2$ μ secs. and the other type of the so-called slow fast coincidence arrangement in conjunction with a twenty-channel or hundredchannel analyzer Resolving time of the latter type was ~ 30 to 40 m.μ secs.

Beta ray spectrometer has been employed to measure the continuous beta ray spectra and the conversion lines of the gamma rays associated with a certain decay Wherever possible, external conversion method has been employed to accurately determine the energies of the gamma rays or if possible the intensities of the unconverted quanta also Secondaries emitted from gold were used for such purposes.

Conversion coefficients of a few gamma rays have been measured by a comparison method wherein the converted and unconverted parts of the gamma ray under-study have been compared against those of another gamma ray whose conversion coefficient is accurately known by some previous experiments. Sources used for such purposes were Cs^{137} and Hg^{203} with gamma rays of 662 and 279-kev respectively The experimental conditions were matched as closely as possible in all such comparison experiments.

A knowledge of conversion coefficient together with K/L or KL+M ratios were used as a guide to determin the multi-polarities of the gamma rays which in turn helped assign the spin values to the excited states emitting the gamma rays in question. Further as far as possible, the relative intensities of various gamma rays have been calculated by using the conversion coefficients and/or the K/L or K/L M ratios in conjunction with the measurements of converted or unconverted parts and the results of coincidence studies.

The isotopes studied include three isotopes of Osmium (Os^{185} Os^{191} and Os^{193}) Gd^{159} and Co^{55}

In the decay of Os^{193} ten gamma rays and seven beta spectra have been detected revealing energy levels in Ir^{193} at 73- 138- 356- 358- 458- and 558-kev The end-point energies of the beta groups in order of decreasing end

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point energies were 1105- 1032 967 749- 717 640- and 547 kev the relative intensities being 67 6 4 2, 3 13 and 5 respectively Energies of the beta groups quoted here are accurate to within 2%

The beta-ray spectrum of Os^{191} was observed and the K/L ratio of the 129-kev transition in its decay was measured as (2.4±0.3) corresponding to a mixture of 54% E2 and 46% M1.

In the study of the decay of Os^{193} gamma rays of energies 75- 122- 158- 233- 593- 643- 718- 750- 870- and 878-kev have been observed revealing energy levels of the residual nucleus Re^{193} at 122 643- 718- 870- and 878-kev Internal conversion coefficients were measured for the 643- 870- and 878-kev gamma rays, the values of αk were determined as (1 14±0.1) X10⁻² (1 1±0.3) X 10⁻² and (5.8±1) X 10⁻³ respectively The value of αk for the 643-kev gamma ray showed that this gamma ray is predominantly E2. A search for positions was made by observing gamma-gamma coincidences with the counters placed at 180° and 90° An upper limit of (4±3) X10⁻⁴ positrons per disintegration could be set.

In the decay of Cd^{160} six gamma rays of energies 56- 80- 136- 225- 305- and 361-kev were observed to decay with the 18-hr half-life of Cd^{160} revealing energy levels in the residual nucleus Tb^{160} at 56- 136- and 361-kev Beta-ray spectrometer studies yielded two principal beta-ray groups with the end-point energies 580±10- and 940±15-kev The coincidence measurements gave evidence of a third beta component with the end-point energy of 880±20-kev, coincident with the 56-kev gamma ray The relative intensities of the three components in order of increasing end-point energy were estimated approximately as 20 6 and 74%, respectively Internal conversion coefficient measurements indicated the 361-kev gamma ray to be an E_1 transition.

In the decay of Co^{55} gamma rays of energies 1410, 937 476, and 417 kev were resolved from observations of the internal conversion lines and the unconverted gamma spectrum No other gamma rays of higher energy decaying with the 18-hr hafl-life of Co^{55} could be observed after careful search of that region using a 4½ in. diameter and 4 in thick NaI (Tl) crystal. Fermi Kurie analysis of the positron spectrum yielded three groups of positrons of maximum energies 1510 1040 and 790 kev having relative intensities as 51 45 and 4 / respectively These measurements revealed energy levels of the residual nucleus Fe^{55} at 937 1410 and 1657 kev

Attempts have been made to interpret the present results on the basis of Nilsson and Cottfried predictions. Results indicate that both the single particle and collective motion characteristics contribute to the excitation spectrum. Apparently much more data is required before a general applicability of such theoretical predictions could be critically examined. There are indications however that these models may not give complete answers to the problem of nuclear structure.

STUDIES ON SLOW COAGULATION

Time of Coagulation by Electrolyte Concentration & the New Equation

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Synopsis of the Work on the Relation Between Electrolyte Concentration and Time of Slow Coagulation in the Light of the Equation

$$C = a + \frac{m. 1/t}{n + 1/t}$$

The first attempt to investigate the relation between electrolyte concentration and the time of coagulation was made by Mathew Murphy and Bouteric who expressed the time of coagulation as a function of concentration, and later on Dumanski formulated the equation $t = ac^n$ where t is the time of coagulation, c is the number of c-cs of electrolyte added to a definite volume of the sol, a and n are constants for the given system. A fairly good amount of work was done by Freundlich, van Arkel, Westgren, Mukerjee and others to test the constancy of the Smoluchowski's factor θ for slow coagulation θ is a function of t, but no correlation between θ and C was known. This has been possible to achieve by combining the Smoluchowski's equation $\Sigma\nu = \frac{\nu_0}{1+t/\theta}$ and the equation $C = a + \frac{m. 1/t}{n+1/t}$ and I have shown that $\theta = \frac{Kn(C-a)}{n-(C-a)}$ where n, m and a are constants which have been interpreted (in previous Communications. That θ can be expressed as a function of (C—a) n, and m is an attribute of the new equation, no reference, however is found of this relation in the earlier contributions on the phenomenon of slow coagulation.

By plotting the concentration, c, of the electrolyte against 1/t, where t is the time of coagulation, it was observed that the curves were hyperbolic in most cases, and in a few cases linear. Such curves were suggestive of the fact that the relation between c and 1/t should be given by some equation which, in general, represents a hyperbola and as a particular case may be reduced to a linear equation. The experimental values of c and 1/t were found to be in satisfactory agreement with the equation $C = a + \frac{m\ 1/t}{n+1/t}$ (1)

where a, m and n are constants. If n is very large compared to 1/t the equation is reduced to the form $c = a + \frac{m}{n}\frac{1}{t}$ which represents a straight line as observed in some cases. In order to verify the general equation I have put it in the form

$$c-a=\frac{m}{nt+1}$$

$$\text{or}\ \frac{1}{c-a}=\frac{nt+1}{m}$$

$$=\frac{n}{m}\ t+\frac{1}{m}$$

which means that a straight line should be obtained in all cases, when $1/(c-a)$ is plotted against t and this has been actually found.

It remains to interpret the constants a m and n If in the general equation $1/t$ is put equal to zero, we get $c=a$. Thus a is that concentration of the electrolyte for which the time of coagulation is infinitely large. In other words a is the critical stability concentration of the electrolyte for the particular sol below which it will not coagulate within measurable time. This is a theoretical confirmation of the observation of Bouterie and other authors that the sols can assimilate small amounts of electrolytes without being coagulated.

Again by taking t to be very small or $1/t$ to be very large the equation (I) gives that $c=a+m$. It means that, with the concentration $a+m$ of the precipitating electrolyte the sol will coagulate immediately Hence m is that excess of the electrolyte concentration over the critical stability concentration, a, which is required to coagulate the sol immediately (rapid coagulation) Thus the concentration $a+m$ is the limit where the region of slow coagulation merges into that of rapid coagulation

Finally a physical significance of n can be obtained by putting t equal to $1/n$ in the general equation (I) which is then reduced to the form,

$$c=a+mn/(n+n)$$

$$\text{or}\ c-a=m/2$$

Thus we can say that n is the reciprocal of the time in which the sol will coagulate when the excess over the critical stability concentration is only half of that required to precipitate the sol immediately It is thus seen that n can be used to denote the effect of smaller fractions, or in other words, it is the sensitivity of the sol towards smaller fractions of the concentration of electrolytes required for the immediate precipitation of the sol. If n is large $1/n$ is small and consequently t is small. So the sol is more sensitive towards smaller fractions.

In my studies on slow coagulation I determined the relation between the concentration of electrolyte and time of coagulation in the light of the new equation, $c=a+\frac{m\left(\frac{1}{t}\right)}{n+\frac{1}{t}}$ by studying the aggregation of As_2S_3, Sb_2S_3, $Fe(OH)_3$ prussian blue, copper ferrocyanide and gold sols with a view to verify the extens

of this equation. The slow coagulation and the times of the same stage of aggregation were determined by measuring the changes in viscosity and light extinction surface leaving method was also used as the general method, where there was difficulty in measuring small changes in viscosity or the optical density was too high. The same stage of coagulation with varying concentrations of electrolytes was obtained graphically by plotting the values of the relative increase of the time of efflux (which is proportional to viscosity) or percentage of extinction against time, and drawing a line parallel to the time axis to make intercepts on the curves which correspond to the concentrations of the electrolytes added to the sol. The points of intersection on these curves enable us to read the times at that particular stage of coagulation, as described earlier The equation $c = a + \frac{m \; 1/t}{n + 1/t}$ by rearrangement of terms reduces to the form $\frac{1}{c-a} = \frac{n}{m} t + \frac{1}{m}$ From this equation it follows that $\frac{1}{c-a}$ is linear with t. Thus, knowing a by extrapolation (and t as described above, $\frac{1}{c-a}$ was plotted against t In every case of As_2S_3, Sb_2S_3, $Fe(OH)_3$, Au, Copper and ferric ferrocyanide sols, the plots, when joined, gave a straight line. This proved the validity of the equation $C = a + \frac{m \cdot 1/t}{n + 1/t}$, by three different techniques—(i) separation of the aggregates from the surface of the medium, (ii) percentage of extinction with time, and (iii) viscosity

Besides the data obtained from the experiments performed by me, this equation was verified by the data obtained by Mookherjee by light transmission through As_2S_3 sol during its slow coagulation with different concentrations of electrolytes. Tezak and Matijevic's data on AgI sol obtained from Time-Tyndallo grams admirably fitted in the equation. The viscosity data of Gann on Aluminium hydroxide and those of Kruyt and Troelstra on silver halide sol by light extinction and, above all, the data of Kruyt and van Arkel on selenium sol by counting the number of the particles under the ultramicroscope, at different intervals of time during its slow coagulation with different concentrations of electrolyte, gave th linear relation between $\frac{1}{c-a}$ and t as required by the equation $C = a + \frac{m \; 1/t}{n + 1/t}$. The constants a, m and n were determined in all cases a and m gave the normal order with the valency of the precipitating ion as should follow from the Schulze Hardy law n is a more complicated factor than a and m in this equation and seems to depend upon several variables of which more adequate knowledge is necessary

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$$\text{or}\ c-a=m/2$$

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the primary particles predominate. In the middle stage this loss of energy is maximum, because both primary and multiple particles having different potentials predominate, and in the end mostly the multiple particles remain to collide with little loss of energy. These changes are depicted by the S-shaped curves. Thus, Burtons concept of the loss of energy produced by the impacts of the primary and the growing multiple particles leads to the conclusion that the inflection point in S-shaped curves is indicative of the stage of coagulation characterised by a transition level in the loss of energy and hence in the rate process of the phenomenon. These deductions lend support to my view that the points of inflection with different concentrations of electrolyte may suitably represent the stage of coagulation. It was confirmed by my observations on the S-shaped curves of the viscosity changes and the extinction curves of AgI sols studied by Kruyt and Troelstra. The times corresponding to the inflection points of S-shaped curves obtained by varying the concentration of the electrolytes were plotted against the values of $\frac{1}{c-a}$. The relation was found to be linear showing the validity of the equation simultaneously. It confers evidence in favour of my assumption that the inflection points should indicate the same stage of aggregation from the view point of kinetics and energy relations and this may be reflected in the variations of physical properties, such as viscosity, extinction and light scattering during the slow coagulation process.

Now the next question of interest was to raise the equation, $C=a+\frac{m. 1/t}{n+1/t}$ from its state of empiricism and find out the ways and means to derive the equation from the basic knowledge of the relation between the number of aggregated and primary particles and the time interval required to reach a certain stage of coagulation, as given by the classical equations of Smoluchowski. Since the Smoluchowski's equation gives the kinetics of the rate of coagulation and the aggregated particles fall under the gravitational force the viscous force of the medium should also be taken for consideration, if we assume that the phenomenon is to be studied by the surface leaving of the mass by a fixed distance x in time, t, required to reach the stage of coagulation when the total number of the particles are Σν and ν_0 was the number of primary particles in the system. The frictional force in the case of falling particles is given by Stokes law. Therefore, it was deemed necessary to combine the results of Smoluchowski' equation and Stökes law to step up the possibilities of deriving my object. The main difficulty in reaching the solution is that there is no reference in the literature by which the Smoluchowski's factor θ can be expressed as a suitable function of the electrolytic concentration which I had to formulate on cogent assumption based on the behaviour of sols with increasing concentrations of the electrolyte vis-a-vis the definition of θ given by Smoluchowski. In brief, the following steps of the derivation might be given to bring in the catch points.

In view of the success achieved in getting remarkable agreement of the equation with the data obtained by the pioneer workers in colloid science, there now remains hardly any plausible ground to doubt that the equation

$C = a + \frac{m.1/t}{n+1/t}$ correctly represents the relation between the concentration of electrolyte and the time of slow coagulation of lyophobic sol, or in other words, the dependence of the time of slow coagulation on the concentration of electrolytes is well expressed by this equation.

An interesting feature of my investigations is the interpretation of the point of inflection in the autocatalytic S-shaped curves of slow coagulation and its identification with the same stage of coagulation. It may not be illogical to emphasise, a priori that the points of inflection in the viscosity—time or extinction—time curves of slow coagulation with different concentrations of an electrolyte represent a characteristic state in the kinetics of the process, where $\frac{d^2x}{dt^2} = 0$

This suggests that during slow coagulation the acceleration of the rate of aggregation measured by viscosity or extinction values is reduced to zero at a certain stage which, a fortiori is governed by the frequency of impacts, size and charge of the particles and also the potential If these are the main variables to govern the process of aggregation, it can be plausibly assumed that the points of inflection obtained by adding different concentrations of an electrolyte would represent that state in a coagulating system which is the same in the sense of kinetics given by $\frac{d^2x}{dt^2} = 0$ and the factors leading to the kinetics of the process, in all probability should attain the same values. Hence I conclude that the points of inflection given by adding different concentrations of an electrolyte may be taken to represent the same stage of coagulation in determining the dependence of the time of coagulation on the concentration of electrolytes.

Any unstable system will tend towards a state with minimum energy content, and this also happens in the case of coagulation of a sol by the addition of electrolytes According to Burton, a colloid particle with its double layer may be considered as a charged condenser in the light of Helmholtz theory It may be proved that, if two charged condensers are brought in contact, there is a loss of electrical energy except when their initial potentials are the same *i.e.* when $\frac{Q_1}{C_1} = \frac{Q_2}{C_2}$ or $V_1 = V_2$ the loss of energy becomes equal to zero. Thus from the above analogy it may be visualised that the loss of energy by the combination of primary particles will be very small or zero because they may be assumed to be at the same potential or very nearly so But the loss of energy increases with the number of multiple particles. So, in the beginning the loss of energy due to impacts is small, because

of colloid from the surface of the medium. Recently B. N. Ghosh has also derived the equation in its present form from Reerinks equation based on the interaction energy consideration, retardation factor and stability ratio of colloidal systems.

Hence it may be claimed that the equation $C = a + \frac{m. 1/t}{n + 1/t}$ has been redeemed of its practical empiricism, as it has now been possible to lay it on a theoretical foundation by two independent methods.

STUDIES IN SOME INDIAN ESSENTIAL OILS*

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The thesis records the investigations on some Indian Essential Oils for their chemical composition. In the course of the work some new compounds were isolated. In cases, where a compound was obtained in a workable yield in pure form, attempts have been made to elucidate its constitution A method for estimation of carbonyls in Vetiver oils has also been standardised. Following is the chapter-wise summary of the thesis.

Chapter I *Essential Oil f Cyperus Scariosus Part I Isolation and Characterisation of the Components*

Rhizomes of Cyperus Scariosus, on steam distillation yield 0.35 % of an essential oil. Dhingra and Dhingra, and Naves and Ardizio reported the presence of the f llowing components () a tricyclic azulenic sesquiterpene, (ii) dl-α-Cyperone (iii) a new α β unsaturated ketone, (iv) a tertiary sesquiterpene alcohol, of the eudalenic type

On re-examination the oil was found to be a complex mixture of many sesquiterpene hydrocarbons and oxygenated compounds The oil was separated, by fractional distillation, into (A) a comparatively lower boiling mixture of tricyclic sesquiterpene hydrocarbons and (B) a complex high boiling mixture, predominantly composed of oxygenated compounds The fraction (A) was composed of two hydrocarbons

(1) A new tricyclic non-azulenic sesquiterpene containing one double bond and possessing the following characteristics

b p 81—82°C/ 1 mm. n_D^{27} 1.5020

D_{30}^{30} 0.9311 α_D −20 05°

The hydrocarbon has been named as Scariosen

(2) A bicycli azulenic hydrocarbon, containing two doubl bonds. Its carbon frame-work has been found to be that of 2 8 dimethyl 5-isopropyl bicyclo (5.3·0) decane

The fraction (B) on repeated fractional distillation and extensive chromatography was found to be composed of

*This is an bstract of the thesis submitted and approved for the Degree of Doctor of Philosophy in Chemistry of the Agra University

(1) A hydrocarbon ($C_{15}H_{24}$) possessing the following properties

b p. 125—130°C (bath)/2 mm.

n_D^{29} 1.5105 α_D + 12 16 to + 13 60°

It is bicyclic in nature as it carries two double bonds. Its Infrared spectrum suggests that the two double bonds are unconjugated and one of them is in the form of a terminal methylene group (characteristic bands at 1639 and 885 cm^{-1}).

(2) A substance, $C_{15}H_{24}O$ having the following properties b. p 120—130°C (bath)/1 mm. n_D^{29} 1.506—1.513 α_D -67 1 to —75.1 The infrared spectrum of the substance exhibited a strong band at 1714 cm^{-1} indicating the carbonyl nature of the substance.

(3) A new $\alpha-\beta$ unsaturated ketone (DNPH m. p. 227.5°C) The ketone has been found to be structurally related to Scariosene, from which it can be obtained by chromic acid oxidation. This relationship shows that the ketone is *tricyclic* and carries one double bond and is not bicyclic carrying two double bonds as suggested by Naves and Ardizio.

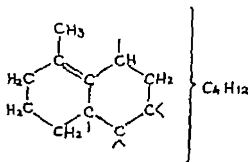
(4) d—α—Cyperone. Naves and Ardizio claim to have isolated racemic α—Cyperone. But during the present investigations d—α—Cyperone was isolated.

(5) A sesquiterpene alcohol possessing the following properties b.p. 121—122°C/2.5 mm. n_D^{28} 1.5110 α_D —14 0 to 16.5° Infrared spectrum of the substance exhibite bands characteristic of a primary alcohol group (1053 cm^{-1}) and a terminal methylene *group* (*887* cm^{-1})

(6) A tertiary sesquiterpene alcohol ($C_{15}H_{26}O$) possessing a camphor like odour Selenium dehydrogenation proves its eudalenic nature and the infrared spectrum suggests the presence of a terminal methylene group (885 cm^{-1})

Chapter II *Essential Oil of Cyperus Scariosus—Part II Studies on the Constitution of the Hydrocarbon Scariosene*

The investigations on the constitution of Scariosene which have been carried out for the first time lead us to the following partial structure for the hydrocarbon It is based on the studies described below



(1) Scariosene ($C_{15}H_{24}$) is a tricyclic hydrocarbon (no. of double bonds 1. Molecular refraction 64.77)

(2) The infrared spectrum of Scariosene does not show any characteristic olefinic absorption, thereby indicating the tetra-substituted nature of the double bond.

(3) Production of Dihydro-scariosene, $C_{15}H_{26}$ (II) on catalytic hydrogenation of Scariosene (I) confirms the presence of one double bond.

(4) Scariosene (I) on oxidation with selenium dioxide yields an alcohol, primary Scariosenol (III) and an aldehyde Scariosenal (IV). This proves the presence of a methyl group adjacent to a double bond $\left(-\overset{\overset{\large CH_3}{|}}{C}=\overset{|}{C}-\right)$

(5) Ozonolysis of the hydrocarbon yields a diketone $C_{15}H_{24}O_2$ (V) which being a methyl ketone (iodoform test) can be oxidised with sodium hypobromite to a keto-acid, $C_{13}H_{21}O$ COOH (VI). The Diketone forms a mono-oxime, a mono-semicarbazone and a mono-2:4 dinitro phenylhydrazone. The above derivatives of the diketone account only for one keto group in its molecule. The second keto group is too hindered to yield a derivative and could be detected through the study of the infrared spectra of the acid VI, oxime VII of the diketone and of the mono-ketone VIII obtained by Huang Minlon reduction of the CH_3CO group in the diketone. Thus ozonolysis confirms the tetra-substituted nature of the double bond, one substituent being a methyl group.

(6) Chromic acid oxidation of Scariosene gives Scariosenone ($C_{15}H_{22}O$) an $\alpha-\beta$ unsaturated ketone (IX). It is identical with the new $\alpha-\beta$ unsaturated ketone found naturally in the higher boiling fractions of the oil. Scariosenone on catalytic hydrogenation yields Dihydro-scariosenone (X). Scariosenone and Dihydro-scariosenone absorb at 1697 cm^{-1} and 1720 cm^{-1} respectively thereby indicating the keto group to be part of a six membered ring. Hence, ring A is six-membered.

(7) The following degradations of the diketone (V) established the structure of ring A in Scariosene.

(i) The diketone on treatment with N-bromo succinimide (NBS) yields the enone XI. The enone on ozonisation gave an aldehyde which could be oxidised with $KMnO_4$ to an acid (XIII) $C_{11}H_{17}O$ COOH.

(ii) The ketone was subjected to Miescher Wettstein degradation, for which it was converted, through Grignard's reaction into a keto-phenyl carbinol (XIV) which was dehydrated to a keto-olefine (XV). The keto-olefine on treatment with NBS and subsequent dehydrobromination afforded the keto diene (XVI) which on ozonisation gave an aldehyde (XVII). The aldehyde

on oxidation with $KMnO_4$ produced the acid (XVIII) which does not give the ferric chloride test for an enol.

(8) The carbonyl frequency of the hindered keto group in the infrared spectra of compounds VI VII VIII XIII and XV lie in the range 1716—1725 cm^{-1} thereby indicating the ring B to be six membered.

(9) Scariosene and many of its derivatives exhibit absorption bands in the region 1006—1039 cm^{-1} assignable to a cyclopropane ring But the presence of a cyclopropane ring could not be proved with certainty by chemical methods.

(10) Scariosene on treatment with NBS yields a heteroannular diene (XIX) which on ozonolysis gives an aldehydic residue (XX)

(11) Scariosene yields unusual oxidation products with peracetic and per benzoic acids With the former Scariosenone (V) and a keto alcohol are obtained. A mechanism, based on some already known analogous cases, has been proposed (Chart III) Among the perbenzoic acid oxidation products an $\alpha\beta$ unsaturated alcohol has been detected.

Chapter III *Essential Oil of the Leaves of Mangifera Indica Part I Isolation of Characterisation of monoterpenes*

Mango (Mangifera Indica) leaves on steam distillation yield 0.015% of an essential oil, which was found to contain 22% monoterpenic hydrocarbons, among which the following were identified 1-alpha thujene, 3-carene ocimene terpinene, camphene and sabinene

Rest of the oil is composed of sesquiterpenes.

Chapter IV *Essential Oil of the Leaves of Mangifera Indica Part II Nature of the Sesquiterpenes*

Two new sesquiterpene hydrocarbons have been isolated from the higher boiling fractions of the oil. These have been named as Mangiferene and Indicene

Mangiferene ($C_{15}H_{24}$) It possesses following properties

b p 85°C/2 mm. n_D^{30} 1 4941 D_{30}^{30} 0 8985 α_D —58°

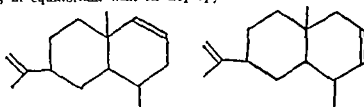
Mol. refraction 66 1 No of double bonds = 2.

The hydrocarbon is bicyclic. The two double bonds are unconjugated and one of them, on the basis of infrared spectrum appears to be part of a terminal methylene group.

On dehydrogenation of Mangiferene with selenium, eudalene is formed On ozonolysis acetone and formaldehyde, the former in predominant excess

over the latter are formed. The non-volatile residue obtained on ozonolysis gives a positive test for aldehyde and a negative test for a methyl ketone

On the above basis, Mangiferene seems to possess one of the following structures, in equilibrium with its isopropylidene form.



Indicene

It possess the following characteristics

b p. 99°C/2 mm. n_D^{30} 1.5001 D_{30}^{30} 0 9115 α_D +26 3°

No. of double bonds 2

Indicene ($C_{15}H_{24}$) like Mangiferene, is b cyclic and eudaleni in nature Spectral data goes to show the unconjugated nature of the double bonds, one of which is in the form of a terminal methylene group

Chapter V *Essential Oil from the Leaves of Murraya Exotica*

Leaves of Murraya Exotica, on steam distillation yield 0 04% of an essential oil. Penfold and Simonsen had identified l-cadinene and bisabolene in the Indian and the Australian oils respectively The presence of neither of these substances could be detected in the oil during the present investigation. The following components were isolated from the oil

(1) *A Sesquiterpene Hydrocarbon* Physical characteristics suggest it to be monocyclic in nature. It contains two double bonds, which are not conjugated. Infrared spectrum suggests the presence of a terminal methylene group

(2) *A Sesquiterpene Ketone* (m. p. of DNPH 144 C) It is bicyclic and carries two double bonds, one of which is part of a terminal methylene group

(3) *A Sesquiterpene Alcohol* It is bicyclic in nature and possesses two double bonds, one of which is in a terminal methylene group On dehydrogenation with selenium, S-Guiazulene was obtained.

Chapter VI *Essential Oil from the Berries of Laurus Nobilis*

The oil was obtained in 3.9-4 1 % yield, by distillation of Laurus Nobilis berries. The oil was found to possess the following composition

Cineole 22 8 %, alcohols (chiefly terpineol) 10.9 %, esters (chiefly methyl cinnamate) 17.2% carbonyls (among which citral has been identified) 11.5%

terpenes (chiefly 1-α-pinene and 1 β pinene) 15.4% sesquiterpenes (one of which is α-caryophllene) 10.1% alkali solubles (among which cinnamic acid has been identified) 9%

The presence of lauric acid earlier reported by Bruhl and Muller could not be confirmed

Chapter VII *Essential Oil from the Leaves of Zanthoxylum Alatum*

The oil was prepared in 0.04% yield by hydro-distillation of leaves in the forest areas of district Garhwal As only 12 gm. of the oil were available for investigations, experiments were carried out mostly by using semi-micro and spot test techniques. Main components of the oil were methyl nonyl ketone (49.21%) alcohols, mainly linalool (19.5%) esters, mainly linalyl acetate (10.67%) and uncharacterised sesquiterpenes (about 15%) Waxy material present in the oil was identified as the paraffin hydrocarbon, tricosane.

Chapter VIII *Estimation of Carbonyls in Indian Vetiver Oils*

Estimation of carbonyl compounds in vetiver oils using bromophenol blue as indicator has always been a problem to the essential oil chemist, because th end point of the titration can not be observed with accuracy

A potentiometric method using a glass electrode has been standardised. The method gives accurate and concordant results. A close study of Zutshi and Sadgopal s method has also been made in the light of the potentiometric method.

DIFFRACTION OF LIGHT BY ULTRASONIC WAVES*

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In 1921 Brillouin predicted that a liquid traversed by compression waves of short wavelengths, when irradiated by visible light, would give rise to a diffraction phenomenon similar to that due to a grating It was nearly a decade after Brillouin's prediction that Debye and Sears (1932) and Lucas and Biquard (1932) independently observed the diffraction of light by ultrasonic waves. Since then, many investigators (Bar Hiedemann Ali, Rytov Sanders, Parthasarathy Korff Becker Nomoto Neumann Bhagwantam Rao Willard, C. R. Rao, Dutta etc) have studied this phenomenon under a variety of experimental conditions and also a number of solutions in different forms have been put forward (by Brillouin, Lucas-Biquard, Raman-Nath Nath Extermann-Wannier Erwin David Rytov Aggarwal, Mertens Nomoto, Bhatia Noble Wagner Phariseau, Rao-Murty etc.)to explain its different experimental aspects A careful study of the literature reveals that most of the experimental work done so far on the measurement of the intensities of the diffraction orders, is confined to low ultrasonic frequencies. Only Bhagwantam-Rao and C. R. Rao have done some work at higher frequencies and have compared their results with David's expression. However the former have estimated the intensities of the diffraction orders only visually and, although the later has measured the intensities with the help of a photomultiplier tube, his studies cover only two aspects of high frequency diffraction viz (i) the dependence of the intensity of the ± 1 orders on the width of the ultrasonic beam, and (ii) the dependence of sharpness of diffraction on the sound wavelength at oblique incidence. Thus no attempt has so far been made to obtain a systematic and precise data about the intensity variations of the different order diffraction lines under various experimental conditions Naturally the various features of the high frequency diffraction as predicted by the more recent expressions of Aggarwal Bhatia-Noble, Phariseau etc have not yet been verified experimentally The need for such a systematic, accurate and thorough investigation of the phenomenon was therefore felt and hence the present work was undertaken.

The thesis describes the work on the precise intensity measurements, with the help of a photomultiplier tube of the diffraction orders with a variety of experimental conditions and the comparison of the results thus obtained with various theoretical expressions.

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Chapter I and II are devoted respectively to the historical surveys of the experimental and the theoretical work done so far. Some of the components of the experimental set up e. g. the r.f oscillators, their power supplies, the matching circuits, the electronically regulated power supply for the photomultiplier tube etc. were assembled by the author. The details about these components, the complete experimental set up used and the method of taking observations appear in Chapter III.

By keeping the wavelength λ ($=5461 \times 10^{-8}$ cms) and the width L ($=1.8$ cms) of the ultrasonic beam constant throughout the experiments the following three factors were changed systematically.

(1) Wavelength λ^* of the sound waves.

(2) Intensity of the sound waves.

(3) *Angle ϕ between the light rays and the sound wavefronts.*

Since λ^* depends on the medium and the frequency used either one or both of these were changed and thus using four different ultrasonic frequencies viz. 7 Mc/sec, 21 Mc/sec, 35 Mc/sec and 49 Mc/sec in different media of propogation variations in the value of $\frac{\lambda}{\lambda^*}$ were obtained in the following eight steps.

S. No.	λ/λ
1	0·0024
2	0·0044
3	0·0075
4	0·0132
5	0·0175
6	0·0218
7	0·0246
8	0·0308

The diffraction pattern was studied at several sound intensities for each of the first five of the above mentioned values of $\frac{\lambda}{\lambda^*}$ and only at one sound intensity for each of the last three values of $\frac{\lambda}{\lambda}$. A broad criterion, viz. the number of orders appearing at normal incidence was taken as a measure of the sound intensity in each of these cases and the corresponding r. f. voltages on the crystal were also measured.

For every value of $\frac{\lambda}{\lambda}$ at every sound intensity the angle of incidence ϕ was changed in several steps by tilting the crystal which was mounted on a

specially designed crystal holder The values of ϕ (which could be changed to a maximum value of $\pm 2°$) were measured accurately by an optical lever system

The experimental data thus collected (given in Chapter IV) have been analysed carefully (Chapter V) summarised separately for different order diffraction lines (Chapter VI—Section A) and finally compared (Chapter VI—Section B) qualitatively as well as quantitatively (wherever possible) with the following theoretical expressions

(I) The expression in the form of Power-Series, for any order diffraction line, obtained by Aggarwal [1-3]

(II) The approximate 'closed expressions, obtained with the assumption that the central order traverses the ultrasonic region without any loss in its intensity (i.e. $I_0 = 1$) for (a) the first order diffraction lines (any angle of incidence) by David,[6] by Rytov [10] by Aggarwal,[1,2,3,4] by Bhatia Noble[8] and by Rao-Murty (b) the second order diffraction lines (any angle of incidence) by David [6] by Aggarwal[3,4] and by Bhatia-Noble,[8] (c) the third order diffraction lines (any angle of incidence) by Aggarwal.[3,4]

(III) The 'closed expressions, obtained without the assumption that $I_0 = 1$ for (a_1) the zero and (a_2) the first order diffraction lines (normal incidence) by Nath [7] for (b_1) the zero and (b_2) the +1 order diffraction lines (any angle of incidence) by Aggarwal [4] by Bhatia Noble[8] and by Phariseau [9] for (c) the −1 order (at the first Bragg angle) by Bhatia Noble[8] and by Phariseau[9] and for (d) the +2 order (at the first Bragg angle) by Bhatia Noble[8] and by Phariseau [9]

The conclusions thus arrived at (Chapter VII) are as follow —

(A) *The First Order Lines*

1 At normal incidence both +1 and −1 orders appear in the diffraction pattern with equal intensity f r th values of $\frac{\lambda}{\lambda} \leqslant 0{\cdot}0246$ When $\frac{\lambda}{\lambda^*}$ is as large as 0 0308 either of these orders appears only at the oblique incidence. The ratio $\left(\frac{I_{+1}}{I}\right)_{\phi=0}$ (i) decreases in general, with the increase in the value of $\frac{\lambda}{\lambda}$ and becomes zero when $\frac{\lambda}{\lambda}$ is 0·0308 and (ii) increases, for the same value of $\frac{\lambda}{\lambda}$ with th increase in the sound intensity except when $\frac{\lambda}{\lambda}$ is as large as 0·0175

All these results can be explained qualitatively either by the expressions mentioned in (I) or II (a) or III (a) or III (b) except the one obtained when $\frac{\lambda}{\lambda}$ is 0·0175 which can be explained qualitatively only either by the expressions mentioned in III (a) or III (b)

2 As the angle of incidence ϕ (and hence $\alpha = \frac{\mu_0 \lambda}{\lambda} \phi$, where μ_0 is the mean refractive index of the liquid) assumes the values greater than zero the intensity of the -1 order (I_{-1}) reduces continuously and that of the $+1$ order (I_{+1}) increases. I_{+1} attains a maxima at $\alpha = 0·5$ in all the cases except when $\frac{\lambda}{\lambda}$ is as small as 0·0024 in which case irrespective of the sound intensity it remains practically constant till $\alpha = 0·5$ With the further increase in the value of α I_{+1} falls off continuously in all the cases.

All these results can be explained qualitatively either by the expressions mentioned in I or II (a) or III (b_2)

3 The maximum value of $\frac{I_{+1}}{I_{-1}}$ occurs at $\alpha = 0·5$ in all the cases except when $\frac{\lambda}{\lambda} \leqslant 0·0044$ The ratio $\left(\frac{I_{+1}}{I_{-1}}\right)_M$ (i) increases in general, with the increase in the value of $\frac{\lambda}{\lambda}$ and for small values of $\frac{\lambda}{\lambda}$ ($\leqslant 0·0044$), is not appreciable, and (ii) decreases, for the same value of $\frac{\lambda}{\lambda}$ with the increase in the sound intensity except when $\frac{\lambda}{\lambda^*}$ is as large as 0.0175.

Of these results (i) can be explained qualitatively either by the expression mentioned in I or II (a) or III (b_2) III (c) but (ii) can be explained qualitatively only from the expressions mentioned in III (b_2) and III (c).

4 The ratio $\frac{(I_{+1})_{\alpha=\frac{1}{2}}}{(I_{+1})_{\alpha=0}}$ (i) increases, in general with the increase in the value of $\frac{\lambda}{\lambda^*}$ It is practically unity when $\frac{\lambda}{\lambda} \leqslant 0·0044$ and infinite when $\frac{\lambda}{\lambda^*}$ is as large as 0·0308, and (ii) increases when $\frac{\lambda}{\lambda}$ is 0·0175 but decreases when $\frac{\lambda}{\lambda}$ is either 0·0075 or 0·0132 with the increase in the sound intensity in each case.

Of these results (i) can be explained qualitatively either by the expression mentioned in II (a) or III (b_2) but (ii) can be explained qualitatively only by the expression mentioned in III (b_2)

5. The ratio $\left(\frac{L_{+1}}{L_{+1}}\right)_{\alpha=\frac{1}{2}}$ (I) increases, in general, with the increase in the value of $\frac{\lambda}{\lambda^*}$ and is infinite when $\frac{\lambda}{\lambda^*}$ is as large as 0·0175 and (II) decreases, for the same value of $\frac{\lambda}{\lambda^*}$ with the increase in the sound intensity

Both these results can be explained qualitatively from the expressions mentioned in III (b_2) and III (d)

6 The sharpness of fall of L_{+1} after attaining a maxima, (i) increases in general with the increase in the value of $\frac{\lambda}{\lambda^*}$ and (ii) decreases for the same value of $\frac{\lambda}{\lambda^*}$ with the increase in the sound intensity

Of these results (i) can be explained qualitatively either by the expression mentioned in I or II (a) or III (b_2) but (ii) can be explained qualitatively only by the expression III (b_2)

7 The sharpness of fall of $\frac{L_{+1}}{L_{-1}}$ after attaining a maxima (i) increases, in general, with the increase in the value of $\frac{\lambda}{\lambda^*}$ and (ii) decreases, for the same value of $\frac{\lambda}{\lambda^*}$ with the increase in the sound intensity

Of these results (i) can be explained either by the expression mentioned in I or II (a) but (ii) cannot be explained by these expressions.

8. The curve $\left(\frac{L_{+1}}{L_{-1}} \text{ versus } \alpha\right)$ ( ) is symmetrical about the centre of the maximum for small values of $\frac{\lambda}{\lambda^*}$ ($=0·0044$ and $0·0075$) (at low sound intensity) and becomes more and more unsymmetrical with the increase in the value of $\frac{\lambda}{\lambda^*}$, and (ii) for the same value of $\frac{\lambda}{\lambda^*}$ with the increase in the value of the sound intensity becomes more and more unsymmetrical about the centre of the maximum.

9 For values of $\frac{\lambda}{\lambda^*} \leqslant 0·0132$ the ratio $\frac{L_{+1}}{L_{-1}}$ becomes unity for large angles of tilt.

This result can be explained qualitatively either by the expression mentioned in I or II (a)

10 The values of $\left(\frac{I_{+1}}{I_{-1}}\right)$ $\alpha=1/2$ obtained experimentally agree quantitatively with those calculated theoretically from an expression derived from the one mentioned in I only in the following cases

(i) $\frac{\lambda}{\lambda^*}=0\ 0024$ ($\rho\S=0\ 8287$)$^{\Delta}$ at all the sound intensities

(ii) $\frac{\lambda}{\lambda^*}=0{\cdot}0044$ ($\rho\S=2{\cdot}698$) only at very low sound intensity i.e. when at $\alpha=0{\cdot}5$, the ±1 order is the highest order in the pattern.

For the values of $\frac{\lambda}{\lambda}\geqslant0\ 0075$ ($\rho\S\geqslant7\ 737$) the experimental and the theoretical values differ widely

11 The values of $\left(\frac{I_{+1}}{I_{1}}\right)_{\alpha=\frac{1}{2}}$ obtained experimentally agree quantitatively with those calculated theoretically from an expression derived from the one mentioned in II (a) only in the following cases

(i) $\frac{\lambda}{\lambda^*}=0{\cdot}0024$ ($\rho\S=0\ 8287$) at all the sound intensities

(ii) $\frac{\lambda}{\lambda^*}=0\ 0044$ ($\rho\S=2{\cdot}698$) only at very low sound intensity

(iii) $\frac{\lambda}{\lambda^*}\geqslant0{\cdot}0246$ ($\rho\S\geqslant91{\cdot}93$)

For the values of $\frac{\lambda}{\lambda^*}\geqslant0{\cdot}0075$ ($\rho\S\geqslant7\ 737$) and $\leqslant0{\cdot}0218$ ($\rho\S\leqslant65\ 65$) the experimental and the theoretical values differ widely

12 The values of $\frac{(I_{+1})\ \alpha=\frac{1}{2}}{(I_{+1})\ \alpha=0}$ obtained experimentally agree quantitatively with those calculated theoretically from an expression derived from the one mentioned in II (a) only in the following cases

(i) $\frac{\lambda}{\lambda^*}=0{\cdot}0024$ and $0{\cdot}0044$ ($\rho\S=0{\cdot}8287$ and $2{\cdot}698$) at all the sound intensities.

(ii) $\frac{\lambda}{\lambda^*}=0\ 0075$ ($\rho\S=7\ 737$) only at low sound intensity

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$$\Delta\ \rho\S=\frac{2\pi\lambda L}{\mu_0\lambda^{*2}}$$

(iii) $\frac{\lambda}{\lambda^*}=0{\cdot}0308$ ($\rho\S=129{\cdot}87$)

For the values of $\frac{\lambda}{\lambda^*}>0{\cdot}0132$ ($\rho\S>24{\cdot}29$) and $\leq 0{\cdot}0246$ ($\rho\S\leq 91{\cdot}93$) the experimental and the theoretical values differ widely

(B) *The Second Order Lines*

1 At normal incidence when the sound intensity is sufficiently high both +2 and −2 orders appear in the diffraction pattern with equal intensity for the values of $\frac{\lambda}{\lambda}\leq 0{\cdot}0132$ When $\frac{\lambda}{\lambda}$ is $0{\cdot}0175$ and the sound intensity is sufficiently high either of these orders appears only at the oblique incidence For values of $\frac{\lambda}{\lambda^*}\geq 0{\cdot}0218$ neither of these appears even at the oblique incidence. The ratio $\left(\frac{I_{+2}}{I}\right)_{\alpha=0}$ (i) decreases in general with the increase in the value of $\frac{\lambda}{\lambda}$ and becomes zero when $\frac{\lambda}{\lambda^*}$ is $0{\cdot}0175$ and (ii) increases, for the same value of $\frac{\lambda}{\lambda}$ with the increase in the sound intensity

All these results can be explained qualitatively either by the expression mentioned in I or II (b)

2. As the angle of incidence ϕ (and hence α) assumes values greater than zero, the intensity of the −2 order (I_{-2}) reduces continuously and that of the +2 order (I_{+2}) increases I_{+2} attains, (i) a maxima at $\alpha=1$ in all the cases except when $\frac{\lambda}{\lambda^*}$ is as small as $0{\cdot}0024$ in which case, irrespective of the sound intensity it remains practically constant till $\alpha=1$ and (ii) two additional secondary maxima at $\alpha=0{\cdot}5$ and 1 5 when $\frac{\lambda}{\lambda}$ is $0{\cdot}0132$ such that $(I_{+2})_{\alpha=1}>(I_{+2})_{\alpha=0{\cdot}5 \text{ or } 1{\cdot}5}>(I_{+2})_{\alpha=0}$ (iii) When $\frac{\lambda}{\lambda^*}$ is 0 0132, with the increase in the sound intensity the maxima diminish in number and the emphasis appears to shift towards the first Bragg angle—the maxima at $\alpha=1{\cdot}5$ and 1 slowly disappear until it occurs only at $\alpha=0{\cdot}5$.

Of these results (i) can be explained qualitatively by the expression mentioned in I or II (b) (ii) can be explained qualitatively only by the expression mentioned in II (b) but (iii) cannot be explained by any of these expressions

3 The maximum value of the ratio $\frac{I_{+2}}{I_{-2}}$ occurs at $\alpha=1$ in all the cases except when $\frac{\lambda}{\lambda}\leq 0{\cdot}0014$ The ratio $\left(\frac{I_{+2}}{I_{-2}}\right)_{\lambda}$ increases,

with the increase in the value of $\frac{\lambda}{\lambda^*}$ and when $\frac{\lambda}{\lambda}$ is small, it is not very appreciable and (ii) decreases, for the same value of $\frac{\lambda}{\lambda}$ with the increase in the sound intensity

Of these results only (i) can be explained qualitatively either by the expression mentioned in I or II (b) but (ii) cannot be explained by any of these expressions.

4 The ratio $\frac{(I_{+1})\ \alpha=1}{(I_{+1})\ \alpha=0}$ (i) increases, in general, with the increase in the value of $\frac{\lambda}{\lambda}$ and becomes infinite when $\frac{\lambda}{\lambda}$ is as large as 0 0175, and (ii) decreases when $\frac{\lambda}{\lambda^*}=0\ 0132$ but increases slightly when it is $\leqslant 0\ 0075$ with the increase in the sound intensity

Of these results only (i) can be explained qualitatively by the expression mentioned in II (b) but (ii) cannot be explained by this expression.

5 The sharpness of fall of I_{+1} and of $\frac{I_{+1}}{I_{-1}}$ after attaining a maxima, (i) increases in general with the increase in the value of $\frac{\lambda}{\lambda}$ and (ii) decreases, for the same value of $\frac{\lambda}{\lambda^*}$, with the increase in the sound intensity

Of these results only (i) can be explained qualitatively either by the expression mentioned in I or II (b) but (ii) cannot be explained by any of these expressions.

6. The curve $\left(\frac{I_{+1}}{I_{-1}} \text{ versus } \alpha\right)$ is unsymmetrical about the centre of the maximum in all the cases

7 For values of $\frac{\lambda}{\lambda} \leqslant 0{\cdot}0075$ when the sound intensity is sufficiently high, the ratio $\frac{I_{+1}}{I_{-1}}$ becomes unity for large angles of tilt.

This result can be explained either by the expression mentioned in I or II (b)

8. The values of $\left(\frac{I_{+1}}{I_{-1}}\right)_{\alpha=1}$ obtained experimentally agree quantitatively with those calculated theoretically from an expression derived from

the one mentioned in I only when $\frac{\lambda}{\lambda} \leqslant 0.0044$ ($\rho \leqslant 2.698$) The agreement is good only when the sound intensity is not high or is such that at $\alpha=1$ the $+2$ order is the highest order in the diffraction pattern

(C) *The Third Order Lines*

1 At normal incidence when the sound intensity is high both $+3$ and -3 orders appear in the diffraction pattern for the values of $\frac{\lambda}{\lambda} \leqslant 0.0075$ When $\frac{\lambda}{\lambda}$ is 0.0132 and the sound intensity is sufficiently high either of these orders appears only at the oblique incidence. For values of $\frac{\lambda}{\lambda} \geqslant 0.0175$ neither of these appears even at the oblique incidence. The ratio $\left(\frac{I_{+3}}{I_0}\right)_{\alpha=0}$ decreases with the increase in the value of $\frac{\lambda}{\lambda}$.

All these results can be explained qualitatively either by the expression mentioned in I or II (c) However although both the expressions I and II (c) expect the third order lines to appear in the pattern with equal intensity experimentally it is observed that there is always an asymmetry in the intensity of these orders even at $\alpha=0$

2. As the angle of incidence ϕ assumes values greater than zero the intensity of the -3 order (I_{-3}) reduces continuously and that of the $+3$ order (I_{+3}) increases. I_{+3} attains (i) a maxima at $\alpha=1.5$ in all the cases, and (ii) three additional secondary maxima at $\alpha=0.5$ 1 and 2 when $\frac{\lambda}{\lambda}$ is 0.0106 and two additional secondary maxima at $\alpha=0.5$ and 1 when $\frac{\lambda}{\lambda}$ is 0.0152. (iii) When $\frac{\lambda}{\lambda}$ is 0.0132, with the increase in the sound intensity the maxima diminish in number and the emphasis appears to shift towards the first Bragg angle–the maxima at $\alpha=1.5$ and 1 disappear and it occurs only at $\alpha=0.5$

Of these esults (i) can be explained qualitatively e ther by the expression mentioned in I or II (c) ( ) can be explained qualitatively only by the expression mentioned in II (c) but (iii) cannot be explained by any of this expressions.

3. The ratio $\frac{I_{+3}}{I_{-3}}$ attains a maxima only when $\frac{\lambda}{\lambda}$ is as small as 0.0024 when it occurs at $\alpha=2.297$ When $\frac{\lambda}{\lambda^*}$ is either 0.0044 or 0.0075, there

is no maxima for $\frac{I_{+3}}{I_{-3}}$ the ratio goes on increasing and becomes infinite at $\alpha=1.98$ and 0.2511 respectively

These results can be explained qualitatively either by the expression mentioned in I or II (c)

4 The ratio $\frac{(I_{+3})\ \alpha=1.5}{(I_{-3})\ \alpha=0}$ increases, in general, with the increase in the value of $\frac{\lambda}{\lambda}$ and becomes infinite when $\frac{\lambda}{\lambda}$ is as large as 0.0152

This result can be explained qualitatively either by the expression mentioned in I or II (c)

5 The sharpness of fall of I_{+3} after attaining a maxima increases, in general, with the increase in the value of $\frac{\lambda}{\lambda}$

This result can be explained qualitatively either by the expression mentioned in I or II (c)

6. The ratio $\frac{I_{+3}}{I_{-3}}$ does not become unity for large tilt angles. The 3 orders generally disappears very quickly

7 The values of $\left[\frac{(I_{+3})}{(I_{-3})}\right]_{\alpha=1.5}$ obtained experimentally agree quantitatively with those calculated theoretically from an expression derived from the one mentioned in I only when $\frac{\lambda}{\lambda}$ is 0.0024 ($\rho\S=2.698$) and the sound intensity is such that at $\alpha=1.5$ the +3 order is the highest order in the diffraction pattern

(D) *The Fifth Order Lines*

1 At normal incidence when the sound intensity is very high, both +5 and −5 orders appear in the diffraction pattern for value of $\frac{\lambda}{\lambda} \leq 0.0044$

The ratio $\left[\frac{(I_{+5})}{(I_{5})}\right]_{\alpha=0}$ decreases with the increase in the value of $\frac{\lambda}{\lambda}$

These results can be explained qualitatively by the expression mentioned in I

2 As the angle of incidence ϕ assumes values greater than zero, the intensity of the +5 order (I_{+5}) increases attains a maxima and then falls off

The value of $\frac{(I_{+8})\ a=2.5}{(I_{+8})\ a=0}$ is smaller for smaller value of $\frac{\lambda}{\lambda^*}$. The fall in I_{+8} after attaining a maxima, is sharper for the larger value of $\frac{\lambda}{\lambda}$

All these results can be explained qualitatively by the expression mentioned in I

References

1 Aggarwal R. R., J. Sc. Ind. Res., 9 B, 110 (1950)
2 Aggarwal R. R., Proc. Ind. Acad. Sci., 31A, 417 (1950)
3 Aggarwal R. R., thesis for Ph. D. (1954) University of Delhi
4 Aggarwal R. R., J. Ph. Radium 20 812 (1959)
5 Bhatia A. B. and Noble W. J. Proc. Phy. Soc. London Part I 220A, 356 (1953)
Part II 220A, 369 (1953)
6 Erwin David., Phys. Zeit., 38, 587 (1937)
7 Nath N. S. N. Proc. Ind. Acad. Sci. 8A, 499 (1938)
8 Phariseau P., Proc. Ind. Acad. Sci. XL IVA, 165 (1956)
9 Rao B. R. and Murty J. S., Z. F. Physik, 152 440 (1958)
10 Rytov S. M., Diffraction de la lumiere par les ultrasons, Actualites Sci. s Ind., 613 (Harmann et Cie Paris 1938)

is no maxima for $\frac{I_{+3}}{I_{-3}}$, the ratio goes on increasing and becomes infinite at $\alpha = 1.98$ and 0.2511 respectively

These results can be explained qualitatively either by the expression mentioned in I or II (c)

4 The ratio $\frac{(I_{+3})\ \alpha=1.5}{(I_{-3})\ \alpha=0}$ increases, in general, with the increase in the value of $\frac{\lambda}{\lambda^*}$ and becomes infinite when $\frac{\lambda}{\lambda^*}$ is as large as 0.0152

This result can be explained qualitatively either by the expression mentioned in I or II (c)

5 The sharpness of fall of I_{+3}, after attaining a maxima increases, in general, with the increase in the value of $\frac{\lambda}{\lambda^*}$

This result can be explained qualitatively either by the expression mentioned in I or II (c)

6 The ratio $\frac{I_{+3}}{I_{-3}}$ does not become unity for large tilt angles. The [illegible] orders generally disappears very quickly

7 The values of $\left[\frac{(I_{+3})}{(I_{-3})}\right]_{\alpha=1.5}$ obtained experimentally agree quantitatively with those calculated theoretically from an expression derived from the one mentioned in I, only when $\frac{\lambda}{\lambda^*}$ is 0.0024 (β=2.698) and the sound intensity is such that at $\alpha=1.5$ the $+3$ order is the highest order of the diffraction pattern

(D) *The Fifth Order Lines:*

1 At normal incidence when the sound intensity is very high, both +5 and -5 orders appear in the diffraction pattern for value of $\frac{\lambda}{\lambda^*} \leqslant 0.0441$

The ratio $\left[\frac{(I_{+5})}{(I_{0})}\right]_{\alpha=0}$ decreases with the increase in the value of $\frac{\lambda}{\lambda^*}$

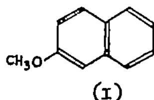
These results can be explained qualitatively by the expression mentioned in I

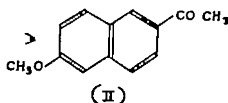
2 As the angle of incidence ϕ assumes values greater than zero, the intensity of the $+5$ order (I_{+5}) increases attains a maxima and then [illegible]

NUCLEAR ACETYLATION IN PRESENCE OF POLYPHOSPHORIC ACID

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In our attempt to synthesise compounds related to sterols, it was found that nuclear acetylation could be brought about readily and effectively by using polyphosphoric acid instead of the conventional reagents. Robinson and Rydon (J Chem. Soc., 1939 1399) prepared 6-acetyl-2-methoxy-naphthalene (II) from 2-methoxynaphthalene (I) using the Friedel-Crafts reaction with acetyl chloride and anhydrous aluminium chloride they had slightly modified the method described by Hayworth and Sheldrick (J Chem Soc., 1934 863) and they obtained a 50 % yield of the product. We have found that by using





polyphosphoric acid (Snyder and Elston, J Amer Chem. Soc., 1955 77 364 see also Sukhdev J I C. S., 1956 33 703 *idem, ibid*, 1957 34, 169) the yield of the ketone (II) has been considerably raised, that is, to 82% Acetylation of anisole which closely resembles 2 methoxynaphthalene (I) has also been carried out under idnetical conditions (see Chemical Reviews, Vol. 58, 1958, p 321) The chief advantage lies in the fact that acetic acid can be directly used as the source of the acylium ion and since the phenol ethers have enhanced susceptibility to electrophilic substitution and are readily soluble in polyphosphoric acid, milder conditions of reaction suffice. The product was identified by a mixed melting point determination with the compound obtained by the method of Robinson and Rydon (*loc cit*) who prepared only the oxime of the ketone. We have prepared several other derivatives of the ketone. The optimum conditions for the reaction were found to be (i) a temperature of 80-85° C, (ii) complete exclusion of moisture, and (iii) a generous proportion of polyphosphoric acid.

EXPERIMENTAL

2 *Methoxy-naphthalene* (*I*)

β-Naphthol was methylated in the usual manner with dimethyl sulphate in presence of aqueous caustic soda. It was crystallised from benzene, giving

colourless plates, m. p 72° C (lit. 72° C) (see Hiers and Hager Organic Syntheses, Collective Vol. I, 59)

Polyphosphoric acid (Sukhdev J I C. S 1955 **32**, 262) —

This was prepared by adding syrupy glacial phosphoric acid (30 ml) in one lot to phosphorus pentoxide (50 gm) and the mixture was stirred vigorously till complete dissolution took place.

6 Acetyl 2-methoxy-naphthalene (II)

2 Methoxy-naphthalene (4 gm) in glacial acetic acid (4 ml) was added to a generous proportion of polyphosphoric acid (48 gms) The mixture was stirred vigorously in complete exclusion of moisture, on a hot water bath at 80-85° C for 2 3 hours, until the colour of the reactants changed to brown-red and ultimately to dark red brown. The mass was poured at once in ice-water giving a fluffy reddish precipitate. It was extracted with ether and the ethereal layer was washed twice with 10% aqueous caustic soda solution The aqueous layer turned black in colour The ethereal layer was separated and dried over anhydrous sodium sulphate Removal of ether gave a yellow solid which on crystallisation from dioxan gave colourless crystalline product, m. p. 105° C (lit. 105° C) Mixed melting point with a specimen prepared by the alternative method did not show any depression. The yield was 82%

Derivatives of the Ketone (II)

The semi-carbazone was prepared in the usual manner Crystallisation from ethanol gave colourless needles, m. p 237 239° C (decomposition) (Found N=15 82 % $C_{14}H_{15}O_2N_3$ requires N=16.34%)

The 2 4-dinitro-phenyl hydrazone was prepared in the usual manner using sulphuric acid Crystallisation from methanol/acetic acid mixture gave red plates, m. p 260° C (Found N=15.2% $C_{19}H_{16}O_5N_4$ requires N=14 73 %)

The picrate was prepared by heating a mixture of the ethanolic solutions of the ketone and picric acid on a water bath for 20 minutes. The yellow solid which separated was crystallised from aqueous dioxan giving yellow fibrous needles, m. p. 102 103° C. (Found N=10.39% $C_{19}H_{15}O_9N_3$ requires N=9 79 %)

WESTERN HIMALAYAN ELM GALLS AND LEAF MINES

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During an expedition to India during September November 1961 to collect western Himalayan elms for hybridization with European species with the main objective of breeding elms resistant to *Ceratocystis ulmi* (Bum.) Moreau Mr H. M. Heybroek collected a number of galls and leaf mines previously unrecorded. Since the relations of the causal organisms with the elm fauna of other regions may throw light on the evolutionary history of *Ulmus*, brief descriptions of these galls and mines are given below

Kaltenbachiella A. (Fig 1) Solitary epiphyllous gall on the basal half of the midrib, causing flexure of the midrib and infolding of the lamina Gall flattened ovoid with the long axis aligned over the midrib $2 \times 1.2 \times 0.8$ cm. Dehiscence via an irregular lateral fissure.

On *U wallichiana* Planch. at Baba Reshi, Kashmir (2200 m.) and Chandan wari, Kashmir (2750 m.)

Dr V F Eastop recovered a cast from one of these galls which agrees closely with *K pallida* (Haliday) The galls very closely resemble the European galls caused by this species It therefore seems likely that the Himalayan specimen either is *K pallida* or a vicariant of it. *K pallida* is widespread in Europe, forming galls on *U carpinifolia* Gled. and *U glabra* Huds. The alternative hosts are *Mentha* spp

Kaltenbachiella B (Fig 2) An assemblage of 2 3 epiphyllous galls along the midrib, causing flexure of the midrib and infolding of the lamina, but not so pronounced as in the previous species Galls depressed globose, 0 7-0 8 cm. in diameter Dehiscence along an irregular circumference leaving a cup-shaped remnant attached to the leaf.

On *U wallichiana* at Verinag, Kashmir (2275 m.)

This species appears to be distinct from the previous one though closely related.

Tetraneura A. (Fig 3) Solitary or sparsely scattered epiphyllous inter venal galls. Gall cucullate, broadly sessile, 0 9 cm. from apex to opening maximum breadth 0.5 cm. Dehiscence via a circular orifice 0.3 cm. in diameter bounded by two revolute lips

On *U wallichiana* at Konam, Uttar Pradesh (2650 m.)

This species resembles but seems not to be identical with both *T ulmifoliae* Baker which occur in Europe and the Far East, and gall 493 of Mani

(1954 1959) *T ulmifoliae* is recorded from *U carpinifolia*, *U glabra*, *U japonica* Sarg., *U parvifolia* Jacq and *U pumila* L., the alternative hosts being Gramineae. Gall 493 was collected from *U wallichiana* in the western Himalayas.

Lithocolletis A (Fig 4) Hypophyllous tentiform mine in the basal half of the lamina. The mine is bounded by secondary nerves on two sides in one case, the midrib constituted a third boundary the remaining side being irregular in the other case, both ends of the mine were irregular Length of mine 0.9-1.3 cm. Epidermis over the mine (lower leaf surface) thrown into an irregular system of longitudinal pleats upper leaf surface over the mine showing the pattern of white spots characteristic of *Lithocolletis* mines.

On *U wallichiana* at Baba Reshi Kashmir (2200 m.) and Kohing, Kulu valley (2150 m.)

This mine bears a close resemblance to that made by the European species *L. schreberella* (Fabr.)

Lithocolletis B (Fig 5) Hypophyllous tentiform mine in the basal half of the lamina The mine occupies most of the area between a pair of secondary veins. Epidermis over the mine (lower leaf surface) with one central longitudinal pleat. Upper leaf surface over mine rather inconspicuously mottled with the white spots characteristic of *Lithocolletis* mines the pair of veins bounding the mine are drawn together slightly so that the dorsal leaf surface is arched.

On *Ulmus* sp near Chaora, Sutlej valley (1500 m.)

Stigmella A. (Figs. 6 and 7) Linear mine. The two specimens of this mine differ in the nature of the frass in the second section of the mine and are therefore described separately In the first specimen (Fig 6) the initial section of the mine is extremely sinuous ca. 1.3 cm. long × 0 01-0 02 cm. wide the frass in segments 0 04-0 18 cm long The junction between the first and second sections is abrupt. The second section is much less sinuous, ca 0.8 cm. long × 0.03-0.04 cm. wide this section is filled with dark brown frass The third section is about 1 2 cm. long and widens gradually until the terminal blotch which is 0 17 cm. across The frass in this section is in the form of granules scattered over the entire width of the mine. In the second specimen (Fig 7) the mine was not completed it resembles the first in the extremely sinuous initial section but differs in that the frass of the second section occupies a central line of uneven thickness. The egg was laid on the upper surface in each case. Larva green.

Found on *Ulmus* sp at Pangot, Uttar Pradesh (2000 m.)

Stigmella B (Fig 8) Linear mine, course erratic, ca. 2 cm. long Mine slender at first, 0 02 cm. wide expanding gradually to a terminal blotch ca. 0 cm. across Frass in early part of the mine made up of segments 0.03 to 0.13 cm. long these segments break up in the latter two-thirds of the mine into granules. The frass occupies the entire width of the mine Egg laid on either upper or lower surface of the leaf.

Found on *Ulmus* sp. near Chaora, Sutlej valley (1500 m.) and Pangot, Uttar Pradesh (2000 m.)

This species bears a close resemblance to the European species *S. ulmivora* (Fologne) which mines *U. carpinifolia*, *U. glabra* and *U. laevis* Pall.

Bucculatrix A. (Fig. 9) Linear mine, course erratic, blistered white in appearance, ca. 1.4 cm. long. Mine at first 0.01 cm. wide gradually widening to 0.04 cm.; it is surrounded by a narrow halo of yellowish brown discoloration. Frass dark-coloured, filling the mine except near its termination. Egg laid on under surface. Larva constructs a small white moulting cocoon on the under surface by the midrib.

On *Ulmus* sp. at Pangot, Uttar Pradesh (2000 m.)

Rhynchaenus A. (Fig. 10) A lobed blotch mine abutting on the leaf margin. Mesophyll completely excavated. Frass in scattered granules, denser towards the centre of the mine. Maximum breadth of mine 1.1 cm.

On *U. wallichiana* at Kolung, Kulu valley (2150 m.)

The larva responsible for this mine appears to be closely related to or identical with the European species *Rh. rufus* (Oliv.) which occurs frequently on *U. carpinifolia*.

Eriophyes (*Aceria*) A. (Fig. 11) Densely aggregated olivaceous galls, frequently in confluent masses or in sinuous lines. Galls most prominent on the under surface of the leaf where they form hemispheres 0.6-1.4 mm. in diameter; they are much less conspicuous on the upper surface.

On *U. wallichiana* at Chandanwari, Kashmir (2750 m.) and Konam, Uttar Pradesh (2650 m.)

Mr. H. H. Keifer recovered mites responsible for these galls; they appeared to be closely related to *E. filiformis* Nal. which produces brownish pustular galls on *U. carpinifolia* and *U. glabra* in Europe.

Only in the last case has the adult organism responsible for the galls or mines described been found and it is much to be hoped that research workers in India may succeed in breeding and describing adult organisms from the structures concerned. However, although the adults are mostly unknown, some cautious speculation on the significance of the findings is permissible.

The affinities of the organisms concerned appear to be mainly with European species. Due allowance must be made for the fact that the elm faunas of China, and to a lesser extent of Japan, are still inadequately explored, but even so the relations of the Himalayan and European elm faunas appear to be remarkably close. The genus *Stigmella* probably reaches its greatest development in Europe where ca. 10 species are recorded on *Ulmus* and one species, *S. ulmivora*, is very similar to *Stigmella* B. Two species mine elms in North America but no Far Eastern *Stigmella* mining *Ulmus* is recorded. Similarly two *Bucculatrix* species are recorded from *Ulmus* in Europe and other undescribed elm-mining

species occur in North America (Hering private communication) no *Bucculatrix* species is known to mine elms in the Far East. *Rhynchaenus* A is very close to or identical with the European *Rh rufus* *Eriophyes* (*Aceria*) A has its counterpart in the European *E filiformis* Three other species of this subgenus produce galls on European elms and one in North America. Gall 37 of Mani (1948, 1959) is produced by *Eriophyes* sp on *Holoptelea integrifolia* Planch. in southern India.

Kaltenbachiella spp. are found on elms in Europe, the Far East and North America. *Kaltenbachiella* A, however appears to be identical with or closely related to the European species *K pallida*. *Tetraneura* spp. are found on elms throughout northern Eurasia (the American species *T graminis* Moodl appear to be more closely related to *Colopha* than to the Eurasiatic *Tetraneura* spp.) and *Tetraneura* A appears to be closely related to the European and Far Eastern *T ulmifoliae* *Lithocolletis* is a genus widely distributed in both temperate and tropical regions. Four species are known to mine elms in Europe, three in Japan (Kumata, private communication) and three in North America. The affinities of *Lithocolletis* A appeared to be with the European *L. schreberella*.

Of species already recorded from Himalayan elms, *Scolytus multistriatus* (Fabr.) also occurs in Europe but not in the Far East (Schedl, 1957) Leaf-rolling aphids have been reported by Mani (1948, 1959) on *U laevigata* Royle and *U wallichiana* in the Himalayas. These galls resemble those caused by *Eriosoma ulmi* (L.) and *E ulmosedens* Marchal, both of which species are widespread on elms in both Europe and the Far East. Other leaf rolling *Eriosoma* species occur in North America. Several elm insects, such as *Abraxas sylvata* (Scop.) and *Discoloxia blomeri* (Curtis) occur in Europe, India and the Far East, but no case appears to have been recorded yet of an insect confined to elms and occurring in the western Himalayas and the Far East but not in Europe.

The simplest inference that seems to follow from these data is that the western Himalayan elms, or some species of them, have been more closely associated in times past with European elm populations than with the far Eastern populations. This is an interesting supposition it is doubtful whether it could have been derived from a comparative study of either the morphology of existing *Ulmus* species or the facility with which interspecific hybrids can be produced.

ACKNOWLEDGEMENTS

Grateful acknowledgements are made to Mr H. M. Heybroek, for making available this collection, Professor E. M Hering, for identifying the mines, Dr V F Eastop, for assistance with the aphid galls, and Mr H.H. Keifer, for his investigation of the eriophyid gall.

SUMMARY

Descriptions are given of galls produced on western Himalayan *Ulmus*

spp., by two species of *Kaltenbachiella*, one of *Tetraneura* and one of *Eriophyes* (*Aceria*) and mines produced by two species of *Lithocolletis*, two species of *Stigmella*, one of *Bucculatrix* and one of *Rhynchaenus*. The affinities of these organisms appear to be mainly with members of the European elm fauna which suggests that some, at least, of the western Himalayan elms may have European rather than Far Eastern affinities.

References

1. Mani, M. S. 1948 : Cecidozoa and zoocecidia from India. *J. roy. Asiat. Soc. Bengal* (*Sci.*) 14 : 27.
2. Mani, M. S. 1954 : Entomological survey of the Himalayas. I. *Agra Univ. J. Res.* (*Sci.*) 3 : 13.
3. Mani, M. S. 1959 : Cecidotheca Indica. *Agra Univ. J. Res.* (*Sci.*) 8 : 91.
4. Schedl, K. E. 1957 : Indian bark and timber beetles I. *Ind. For. Rec.* (*Entomology*) 9 : 165.

Legend to Figures

Western Himalayan elm galls and leaf mines. 1. *Kaltenbachiella* A; 2, *Kaltenbachiella* B; 3. *Tetraneura* A; 4. *Lithocolletis* A; 5. *Lithocolletis* B; 6 and 7. *Stigmella* A; 8, *Stigmella* B; 9. *Bucculatrix* A; 10. *Rhynchaenus* A; 11. *Eriophyes* (*Aceria*) A.

species occur in North America (Hering, private communication) no *Bucculatrix* species is known to mine elms in the Far East. *Rhynchaenus* A is very close to or identical with the European *Rh. rufus* *Eriophyes* (*Aceria*) A has its counterpart in the European *E. filiformis* Three other species of this subgenus produce galls on European elms and one in North America Gall 37 of Mani (1948, 1959) is produced by *Eriophyes* sp on *Holoptelea integrifolia* Planch. in southern India.

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SUMMARY

Descriptions are given of galls produced on western Himalayan *Ulmus*

OPTICAL ROTATORY POWER & CHEMICAL CONSTITUTION*

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The author has described in this thesis the effect of certain groups on optical rotatory power in some optically active derivatives of camphor β-sulphonic acid, brucine and strychnine The optically active derivatives prepared and studied in this thesis are —

1 *Derivatives of optically active camphor β- sulphonic acid* —

(i)	Ammonium—	(D- and L-)	Camphor	β-	sulphonate.
(ii)	Methylammo—		"	"	
(iii)	Di-methylamino—	"			
(iv)	Ethylamino—	"	"	"	"
(v)	Ethanolamino—		"	"	"
(vi)	Iso-propylammo—	"	"	"	
(vii)	n-Amylamino—	"		"	"
(viii)	Allylammo—	"	"	"	"

2. *Derivatives f optically active strychnine* —

(i)	Strychnine Benzoate.			
(ii)	Strychnine	-o-	Chloro	Benzoate.
(iii)		-m-	"	"
(iv)		p-	"	
(v)	Strychnine	-o-	Bromo	Benzoate.
(vi)	"	-p-		
(vii)	Strychnine	-o-	Amino	Benzoate.
(viii)	"	-m-	"	"
(ix)	"	-p-	"	
(x)	Strychnine	-o-	Methyl	Benzoate.
(xi)	"	-m	"	
(xii)		p-		
(xiii)	Strychnine	-o-	Nitro	Benzoate
(xiv)	"	-m-	"	"
(xv)		-p-	"	"

* This is an abstract of the thesis submitted and approved for the Ph. D degree of the Agra University for the year 1960.

3 *Derivatives of optically active brucine* —

(i) Brucine salt of Isonitroso—Acetophenone.
(ii) " Isonitroso Diethyl Ketone.
(iii) " α Chloro- α Isonitroso—Acetophenone.
(iv) " -p- Methyl-Acetophenone.
(v) " " -p- Methoxy Acetophenone.
(vi) " " p- Chloro-Acetophenone.
(vii) " -αBromo -α Isonitroso Acetophenone.
(viii) " -p- Methyl-Acetophenone.
(ix) -p- Methoxy-Acetophenone.
(x) p- Bromo-Acetophenone.
(xi) " Methyl-β-Naphthyl Ketone.
(xii) -αBromo -α Nitroso Methyl Ethyl-Ketone.
(xiii) Diethyl Ketone.

The total number of compounds prepared and studied is 36 out of which 33 are new compounds prepared and studied for the first time. Out of these compounds in the case of derivatives of Reychler's acid both D- and L- isomers have been prepared and studied. After characterising the new compounds their optical rotation for 12 wave lengths in the visible region in different solvents has been studied. The solvents used are given in Table 1 below

TABLE 1

Optically active compounds	Solvents used for study of optical rotation.
1 Ammonium (D- and L-) Camphor β- sulphonate	Water Methyl Alcohol Ethyl Alcohol.
2. Other derivatives of Reychler's acid	Water Methyl Alcohol Ethyl Alcohol Chloroform Pyridine.
3 Derivatives of brucine	Chloroform Pyridine.
4 Derivatives of strychnine	Chloroform Pyridine.

In each case the optical rotation data was analysed both graphically and mathematically and rotatory dispersion equations were calculated. All the compounds studied in this thesis exhibited simple dispersion and their rotatory

power can be expressed by Drude's one term equation of the type $[\alpha] = \frac{K}{\lambda^2 - \lambda_0^2}$ where $[\alpha]$ is specific rotation for wavelength λ and K and λ_0 are constants.

The effect of various groups on the optical rotatory power of derivatives of camphor β- sulphonic acid, brucine and strychnine has been discussed The groups thus studied in the case of different series of compounds are given in Table 2 —

TABLE 2

Derivatives of	Groups studied
1 Camphor β-Sulphonic acid	$-CH_3$, $-(CH_3)_2$, C_2H_5, $CH-CH_2OH$, $-CH(CH_3)_2$, $-C_3H_7$, $-CH_2-CH=CH_2$,
2. Strychnine	$-Cl$, $-Br$, $-NH_2$, $-CH_3$, $-NO_2$
3 Brucine	$-CH_3$, $-OCH_3$, $-Cl$, Br, $C_{10}H_7$, C_6H_5

In each series of compounds the effect of solvent, concentration temperature and wave length of light used has also been discussed

The case of strychnine p- nitro benzoate is remarkable as it shows [α] [illegible] = −97 00° in pyridine and zero rotation in chloroform. This case has been studied in detail by using mixed solvents and a probable explanation has been suggested.

Certain groups studied are common to some of the series. These groups are $-CH_3$, Cl, $-Br$ Their effect on optical rotation in different series of optically active derivatives has been compared.

The studies summarised above are found to lead to the following general conclusions —

1 In any study of the effect of the groups on optical rotatory power it is necessary to take into account the effect of factors such as concentration of the solute, nature of the solvent used, temperature at which observations are made and the nature of the rotatory dispersion which the compound exhibits. Comparisons are valid only if they are done under comparable conditions.

2 When the author compared the effect of groups in each of the three series of optically active derivatives mentioned earlier it was noticed that the polarity of a group appears to have a marked effect on optical rotatory power. This effect, however is sometimes masked by the nature of the solvent employed. A polar group in different polar solvents can exhibit different type of effects.

3 When a comparison is made of the effect of a group in one series of optically active derivatives with the effect of the same group in another series of optically active derivatives it is found that though in general the effect is qualitatively similar but sometimes it may not be so.

BIOMICROSCOPICAL STUDY OF THE ELASTICITY OF CONJUNCTIVAL VESSELS IN HYPERTENSION BY ADRENALINE METHOD*

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Introduction

General interest in the study of hypertension has gained much ground all over the world during the last few years, as it constitutes one of the leading causes of death. Early diagnosis of it is, therefore of immense importance because patients treated at this stage can be saved from the various permanent damages that would otherwise occur to the important vital organs in the body

Ophthalmoscopy has no doubt won great recognition as a measure in the diagnosis of hypertension but unfortunately the diagnosis with ophthalmoscopy is only possible in the later stages of the disease. Therefore attempts were made to find out some measure for early diagnosis of sclerosis People proposed to study directly the changes into the blood vessels through a biomicroscope and the site chosen was the bulbar conjunctiva. Mathurs K. N and S N (1957) studied the pattern and elasticity of the conjunctival vessels in normal and hypertensive cases by using Priscol—a vasodilator drug This study definitely made a useful contribution for the early diagnosis of sclerosis, but the method caused great inconvenience to the patients, as it took longer time for the completion. Hence the necessity for another alternative method which would be less time-consuming and hence less tiring to the patient was felt. Adrenaline, being a short acting drug it was felt, would be less time-consuming and thus less tiring to the patient and was therefore tried to determine the elasticity of vessels in place of Priscol in this series of cases.

Material and Methods

A total number of 45 cases were examined, out of which 25 were normal and 20 hypertensive

Biomicroscopical Examination of the Conjunctiva

It was carried out into one of the cubicles of the dark room which was fitted with a glass-topped adjustable table housing the corneal microscope and having an adjustable chin and head-rest, two adjustable stools, a 15-watt lamp, a mirror placed at a suitable angle and a modified T D C projector

The patient was then made to sit on one of the stools for examination with hands on the top of the table. Because of the difficulty in fixing the eyes for

*This is an abstract of M. S (Opth) Thesis of the Agra University

examination the patient was asked to fix his eyes on the image of a 15-watt lamp into a mirror which was placed in front and to one side of the patient. The bulb was placed behind the patient diagonally opposite to the mirror. When the eye was fixed in this way a greater area of the bulbar conjunctiva on the temporal side was exposed for examination.

Corneal Microscope

The binocular corneal microscope with 10X oculars were used along with No 4 (Zeiss) objectives In one of the oculars a circular glass piece graduated to 1/100 of a centimeter was inserted and kept in position by a wire ring. The milliscale was used for measuring the diameter of the blood vessels. In measuring a blood vessel, the eye piece having the micrometer scale was rotated in such a fashion that the markings of the scale were parallel to the course of the vessel Direct reading on the scale gave the width of the vessel.

Light Source

The light source was a T D C. projector on which was fitted a special metallic cylinder housing 3 convex lenses of 13 dioptres power and a cooling chamber

Photographs

Photographs of the conjunctival vessels were taken by a 35 m.m. PRATICA camera. It could be fixed to one of the oculars of the corneal microscope after taking out its lens.

Drug

The drug used for causing vasoconstriction was Adrenaline Hydrochloride

Fundus

Fundus examination was done with oculus visuoscope after dilating the pupils with 2 % homatropine

OBSERVATIONS

All cases were examined under three different age groups —

1 Upto 40 years,
2. Between 40 and 50 years, and
3 Above 50 years.

Blood vessels of the diameter of 0.2 0.25 and 0.3 m. m. were observed in all the normal cases. In the hypertensive series besides blood vessels of 0 2, 0.25 and 0 3 m.m diameters, vessels of 0 15 m. m. diameter were also seen in a few cases belonging to the first and second age groups.

Pattern of Conjunctival Vessels

Normal—In the normal series, cases belonging to the 1st age group did not show any change in the pattern of their vessels.

In the second age group, changes in the pattern of conjunctival blood vessels were observed, and tortuosity was far more commonly seen than loops.

In the third group, changes in pattern of conjunctival blood vessels were far more common and tortuosity was more frequetly observed than loops.

Hypertensive Series

In hypertensive series tortuosity and loops in the conjunctival vessels were far more commonly seen than in the normal cases of the same age groups.

Amount of elasticity of the conjunctival vessels as evident by the action of Adrenaline Hydrochloride

Normal Series

It was observed that in all cases irrespective of their age groups constriction in the blood vessels started 2 minutes after the instillation of drug into the conjunctival sac.

In the first age group the amount of maximum constriction in the vessels, observed after the instillation of drug was 45 7 ± 9.2 / In 75% of the cases, they returned to their original diameters within 20 to 24 minutes

In the second age group the amount of maximum constriction observed in the blood vessels was 39 8 ± 7.2 % and they returned to their original diameters within 24 to 28 minutes.

In the third age group the maximum constriction observed was 39 1± 12.6% and the vessels returned to their original diameters within 30 to 40 minutes.

Hypertensive Series

In all the hypertensive cases also irrespective of their age groups, constriction started in the vessels 2 minutes after the instillation of drug into the conjunctival sac.

In the first age group the amount of maximum constriction in the vessels, observed, was 36 4 ± 12.7% as against 45.7 ± 9.2 % observed in the normal cases of the same age group. The vessels also took a longer time of 28 to 30 minutes to return to their original diameters as against 20 to 24 minutes taken by normal cases of the same age group

In the second age group the amount of maximum constriction observed was 34.3 ± 12.4 % as against 39.8 ± 7.2 % that was observed in the normal cases of the second age group. In majority of the cases the vessels took 26 to 34 minutes as against 24 to 28 minutes taken by normals of th same age group to return back to their original diameters.

In the third age group the amount of maximum constriction observed was 34.0 ± 12.7 % as against 39 1 ± 12.6 % observed in the normal cases

of the 3rd age group The vessels took 34 to 50 minutes to return back to normal as against 30 to 40 minutes taken by normals of the same age group.

RESULTS

1 The pattern of the conjunctival blood vessels changes with age. As the age advances, tortuosity and loops become more and more common in the vessels.

2 The elasticity of the conjunctival blood vessels progressively decreases as the age advances.

3 In hypertension the pattern of the conjunctival blood vessels is very *much changed. Tortuosity and loops in the vessels become very much* common in the younger age group

4 In hypertension the elasticity of the conjunctival blood vessels decreases.

5 It is observed that in cases showing blood pressure above normal, *the elasticity of the* conjunctival blood vessels is diminished. While in cases showing systolic blood pressure above 200 mm. Hg the elasticity is found to be markedly diminished.

6 *In hypertension*, changes in the pattern and elasticity of the conjunctival blood vessels are present even before the appearance of any hypertensive changes in the fundus as deducted by ophthalmoscope. While the conjunctival vessel findings are always present in those cases showing positive fundus picture of hypertensive retinopathy It is also noted that in those cases which show severe fundus changes of hypertensive retinopathy the elasticity of the conjunctival vessels is also markedly diminished.

DISCUSSION

This study was undertaken with the idea of finding out a method for diagnosing hypertension in early stages by studying a change in the pattern and elasticity of conjunctival vessels. To test the latter a short-acting vasoconstrictor drug Adrenaline Hydrochloride, was used with two ideas —

1 To confirm the findings of Mathurs *K.N and S N who have found* out a change in the pattern and in the elastic ty of conjunctival each in hypertension by using Priscol, a vasodilator drug

2 Secondly being a short-acting drug it will take lesser time for completion of the experiment and thus would be less tiring to the patient a great advantage on the previous work

It was observed that in normal cases with the advancement of age the pattern of the conjunctival vessels also changed as was evident by *increased*

tortuosity and loops in the older age groups Secondly the elasticity of the conjunctival vessels gradually diminished with advancement of age as was evident by lesser amount of maximum constriction and delayed return to normal in older age groups after the instillation of Adrenaline Hydrochloride into the eye.

In hypertension the change in the pattern of conjunctival blood vessels was not only observed in older age groups, but also in younger age group. Moreover the elasticity of these vessels as tested by Adrenaline Hydrochloride was found to be very much diminished showing marked sclerotic changes in conjunctival vessels during hypertension All these findings go in collaboration with that of Mathurs, K. N and S N and Lack.

The importance of this method becomes still more because firstly it is less time-consuming and thus less tiring to the patient and thus of more practical utility in day-to-day practice, and secondly it is as accurate a method as the previous one where Priscol was used for studying the elasticity of conjunctival vessels.

Conclusion and Summary

This study brings a new light to the gloomy world of hypertension. It will be now possible to diagnose early cases of hypertension with this method by directly visualising the pattern of the conjunctival blood vessels and by measuring their elasticity with the help of a short vasoconstrictor drug Adrenaline hydrochloride.

It has now been proved beyond doubt by both dilatation and constriction methods of the conjunctival vessels with a vasodilato drug like Priscol and a vasoconstrictor drug like Adrenaline hydrochloride that the elasticity of the conjunctival vessels in hypertension is diminished due to sclerotic changes in them.

Diagnosing an early case of hypertension by vasodilatation method is not very practicable because it is much time-consuming and very tiring to the patient This second method in which we have used Adrenaline hydrochloride is more advantageous because being less time-consuming is less tiring to the patient and thus becomes more practicable in day-to-day practice Secondly it is as accurate a method as the dilatation one of finding out the sclerosis with Priscol.

A total number of 45 cases were examined in this study Twenty five were normal ones and the rest twenty were suffering from essential hypertension. Normal cases were examined under three different age groups in order to set up a definite pattern of th conjunctival vessels and a standard of their elasticity so that they may act as a control for the hypertensive cases. Fund

was examined in each case. Later on hypertensive cases were examined in the same way as the normal, and their fundus were also examined.

It was found out that sclerosis in conjunctival vessels in hypertension, as evident by a change in their pattern and a diminution in their elasticity to vasoconstrictor drug Adrenaline hydrochloride, was detectable earlier than the sclerosis in the fundus vessels by a ophthalmoscope.

ACTIVITIES OF PLANT NUTRIENT IONS (CATIONS) IN VARIOUS TYPES OF SOILS CLAYS AND CLAY MINERALS IN RELATION TO THEIR AVAILABILITY TO PLANTS*

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This investigation deals chiefly with the availability of potassium on the basis of its energy relationships in a manner similar to the one used successfully in recent years for determining the available soil moisture. This treatment appears to have certain advantages over the usual methods currently used for estimating available potassium. The activities of the ions are connected to the free energy changes by means of the following thermodynamic relationships as introduced by Lewis —

$$\bar{F}_i = \left[\frac{\partial F}{\partial n}\right]_{t\ p\ n_i} \quad (1) \qquad \bar{F}_i = \bar{F}_i^{\circ} + RT\ ln\ a_i \quad (2)$$

$$\phi = \phi + RT\ ln\ a_i \quad (3) \quad \text{and } f \times c = a \quad (4)$$

where $\bar{F}_i$ is the partial molar free energy ϕ the total potential, f the activyti coefficient of the ion, c the conc. of the ion and a the total activity of the ion concerned n_i the number of mols of ion I.

Sand culture experiments with wheat, tobacco, maize and mung at a potassium concentration of 0.25 0.5 1 2 and 4 me./lit. and a corresponding calcium concentration as to make the total concentration in all the five culture solutions equal to 10 me./lit. were conducted. These have shown that the optimum upper limits in solution for Ca/K ratios expressed in equivalents are 9 4 4 and 9 respectively for different crops mentioned above The corresponding upper limits for Ca/K in plant composition are 0.55 for the whole plant of wheat, 2.07 for the leaves of tobacco, 0.51 for the whole plant of maize and 6.27 for the whole plant of mung harvested at the time of first picking of mung and at the flowering stage in the rest of the crops. These values agreed with the corresponding data published in literature within the limits of accuracy of these experiments. The optimum potassium percentage in wheat plants is found to be 0.82 per cent as against 0.83 per cent reported by Hoffmann. For tobacco leaves the lower limit of potassium percentage was found to be 2.57 in agreement with the value of 2.79 reported by Lagatu & Maume. In the case of maize plant the value found for potassium deficiency limit is 2 73 per cent as against 1·99 reported by Stanford et al.

Since the method of diagnosing nutrition l deficiencies from plant

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This is an abstract of the thesis submitted and approved for the Ph. D degree of the Agra University for the year 1960.

composition is not useful for the current crop the usefulness of soil test methods as judged by their correlation with crop responses to potassic fertilizers using paddy crop in field experiments has been investigated.

The coefficient of correlation on 40 soils from 4 blocks was -0.057 the smallest value, for 1% citric acid -0.5187 for diffused postassium, -0.195 for 0.5N HNO_3 (1 hour heating method) -0.2440 for 0.5N HNO_3 (½ hour shaking method) -0.234 for Ammonium Acetate, -0.353 (significant at 5% level) for the degree of potassium saturation, -0.365 (significant at 5% level) for potassium adsorption ratio of twice saturated extract, -0.381 (significant at 2% level) for Morgan's extractant and -0.650 (significant at 1% level) for potassium adsorption ratio calculated (rapid method). When these soils were separated block wise none of the methods used gave significant correlation for all blocks excepting potassium adsroption ratio calculated. The inability of the usual soil test methods to correlate with crop responses in different blocks of soils as compared to potassium adsorption ratio (calculated) values indicates the necessity to determine the thermodynamic availability of soil potassium which the potassium adsorption ratios measure in order that significant correlation could be obtained on a wide range of soils.

Since the exchangeable potassium in any colloidal system remains undisturbed in equilibrium solutions irrespective of the activities of potassium in the latter so long as the activity of potassium in the solution is balanced by a corresponding activity of a divalention so as to make the ratio of the activity of potassium to the square root of the activity of divalent cation constant. This ratio termed the potassium adsorption ratio or the constant of the ratio law function has been interpretted in this investigation as the tension against which the exchangeable potassium of the soil has been held. This makes the negative logarithm of this tension when multiplied by RT related to the free energy decrease associated with soil potassium. Woodruff has interpretted RT ln potassium adsorption ratio as the free energy decrease in the replacement of potassium by an equivalent amount of divalent cation

Besides a method similar to the saturated extract of Woodruff, another one more rapid but approximate and three other thermodynamically more satisfactory methods of determining potassium adsorption ratio of soils have been described. These are calculated from either degree of potassium saturation or diffused potassium of natural soils in conjunction with the curves connecting potassium adsorption ratio of equilibrium solution either with degree of potassium saturation or diffused potassium as the case may be of soil samples equilibrated with solutions of various potassium adsorption ratio values. The other method called ± method, was the selection by trial and calculation of a solution with such a potassium adsorption ratio that would not undergo any change on equilibration of the soil with the solution.

A relatively rapid and approximate method used was to calculate the potassium adsorption ratio of the natural soil from the exchangeable potassium

assuming a direct proportionality between exchangeable potassium of the equilibrated soil and the potassium adsorption ratio of the equilibrium solution used as a standard for the purpose.

There was generally a good coefficient of correlation between the values obtained by these various methods. The potassium adsorption ratio by degree of potassium saturation and ± method however appeared thermodynamically more satisfactory while the potassium adsorption ratio calculated has the advantage of being more rapid than the others.

Potassium adsorption ratio (K. A. R.)of the plants grown in sand culture has been determined and found to be 0.308×10^{-2} for wheat, 0.715×10^{-2} for paddy 1.45×10^{-2} for maize, 2.04×10^{-2} for mung and 2.87×10^{-2} for tobacco. These values showed a positive statistically significant correlation at 2 % level with cation exchange capacities of roots which are 9.2 me. for wheat 14.2 me. for paddy 14.5 me. for maize, 35.5 me for mung and 49.5 me. for tobacco per 100 gms. of dry matter. The values of K.A.R of plants work out to be energies of release of potassium by divalent cations (calcium and magnesium) in equivalents of the order of 3242 cals. for wheat, 2927 cals. for paddy 2509 cals. for maize, 2291 cals. for mung and 2114 cals. for tobacco. This indicates that other things remaining equal the need for potassic fertilizers increases in the order tobacco > mung > maize > paddy > wheat.

The potassium adsorption ratio values of plants was found to indicate the relative potassium needs of plants. The available data gave a preliminary indication that a crop will respond to potassic fertilizers on a soil only when the K. A. R. of the soil is lower than that of the plant. The average of calcualted potassium adsorption ratio values for the soils which did not respond to potassic fertilizers with paddy crop was 0.719×10^{-2} which is very near to the equilibrium K. A. R. of paddy plant. Similarly the soils with wheat crop also indicated potassium sufficiency at a K. A. R. value between 0.190 and 0.323 while equilibrium K. A. R. value of wheat plant was 0.308.

(6) The potassium adsorption ratio in equilibrium solution is generally related to the exchangeable cations contents by means of various ion exchange equations. Some of the important ones proposed by Kerr, Vanselov, Krishnamoorthy and Overstreet, Gapon and one based on Donnan membrane equilibria have been used in this investigation to see the constancy of the equilibrium constant with various types of soils, clay minerals and oxides, varying the concentration and K. A. R. of the equilibrium solutions. A statistical examination of these equations has shown that the Gapon's equation was most suitable for the materials studied. It was also found that the equilibrium constant decreases with the increase of concentration and K. A. R. of the equilibrium solution.

On the basis of this finding and the following three assumptions, the single ion activities in the soils have been calculated. One is that only potassium,

calcium and magnesium are the dominating cations in the exchangeable phase the other that the activity coefficients of calcium and magnesium are nearly the same and the third is that the movement of water between exchangeable and solution phases is such that the osmotic pressure is the same on both the phases. The feature of the results so obtained is that the activities of potassium and divalent cations show Wiegner Pallmann effect.

When the bonding energies of potassium are calculated and curves are drawn connecting the free energy decreases and degree of potassium saturation, these indicate that potassium is bound more firmly to kaolinite than to montmorillonite at lower degrees of potassium saturation and is in accord with the inverse ratio law The position of illite is higher than those of other clay minerals In the case of Pusa and Chalkudi soils, there seems to be an intermediate range of D K. S where the free energy change involved is more or less constant. In general the curves show that the bonding energy decreases with the increase of degree of potassium saturation in the range studied. This being more steep in the case of materials of higher cation exchange capacities. The curve connecting the ratio of activity coefficients of divalent cations to potassium with the degree of potassium saturation could be generally used for the physico-chemical characterisation of the soils in terms of the behaviour of reference clay minerals and oxides

However useful the activities are as shown above, they are applicable only in cases where the above assumptions are valid while the determination of the ratio of activities of potassium to divalent cations raised to reciprocal of their valency is not subject to these limitations, easier to determine and useful in evaluating potassium in soils as measured by crop responses to potassic fertilizers.

PHYSICO-CHEMICAL STUDIES OF ALKALOIDS AND OTHER BASES PREPARATION OF JABORANDI ALKALOIDS AND STUDIES ON SEPARATION OF ALKALOIDS FROM DRUGS*

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The present investigation has been divided in three parts —

PART I VEGETABLE ALKALOIDS

In this part a brief introduction to the alkaloids has been given This includes, occurance, distribution and mode of action, function in plants, nomen clature and classification general properties, general alkaloidal reagents general methods for determination of structures and isolation of alkaloids.

PART II SYNTHESIS OF THE ALKALOIDS OF JABORANDI

The earlier methods for the preparation of jaborandi alkaloids, as described in the literature, have been found to be long and combersome In part II of the present work an attempt has been made to develop new route for the preparation of jaborand alkaloids and their degradation products. For the sake of convenience this part has been further divided into sub-sections as described below

Sub-section A *Alkaloids of Jaborandi*

In this sub-section a historical survey of jaborandi alkaloids, their pharmacological action, establishment of their constitution and also the constitution of their degradation products and the synthesis of the alkaloids and their degradation products, as has been described in the literature, is given

Sub section B *Synthesis of Lactonic Acids*

In the earlier methods, for the preparation of r-lactonic acids (the degradation products of jaborandi alkaloids) Preobrashenski and others have used substituted succinic acids and from these homo lactonic acids were prepared through a long procedure, in which reagents such as diazomethane has also been used. In another method Dey prepared homo lactonic acids from substituted hydroxy glutaric acids. In the present investigation these r-lactonic acids were first prepared from substituted butyri acids. For this purpose glycerol (1) was converted to glycerol a, -dichlorohydrin (2) by the method of Contant et al. The latter by the method of Clarke and Hartman was converted to epichloro-hydrin (3) which when treated with ethyl alcohol gave

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a-chloro-hydrin r-ethyl ether (4) This was then boiled with potassium cyanide in alcoholic medium. a-cyanohydrin r-ethyl ether (5) thus prepared upon dehydration with phosphorus pentoxide produced 1-cyano 3-ethoxy ?-propene (6) The latter was then condensed with diethyl malonate. Ethyl 1-carbo-ethoxy 2-ethoxy methyl 3-cyano butyrate (7) thus obtained was hydrolised to homo pilosinic acid (8) in sufficient yields.

$$CH_2OH.CHOH\ CH_2OH \longrightarrow CH_2Cl.CHOH.CH_2Cl \longrightarrow ClCH_2\ \underset{O}{CH{-}CH_2} \longrightarrow$$

(1) (2) (3)

$$ClCH_2\ CHOH.CH_2OC_2H_5 \longrightarrow CNCH_2.CHOH.CH_2OC_2H_5 \longrightarrow CN\ CH{=}CH.CH_2OC_2H_5$$

(4) (5) (6)

$$CH_2(COOC_2H_5)_2 \xrightarrow{NaOC_2H_5} \begin{array}{l} CNCH_2CH.CH_2OC_2H_5 \\ \quad | \\ \quad CH(COOC_2H_5)_2 \end{array} \longrightarrow \begin{array}{l} CH_2 \text{———} CH.CH_2COOH \\ | \qquad\qquad\quad | \\ CO-O-CH_2 \end{array}$$

(7) (8)

In an attempt to generalise this method for the preparation of other homo lactonic acids, dl-homo pilopic acid (10) was prepared by condensing ethyl 1-carboethoxy 2-ethoxy methyl 3-cyano butyrate (7) with ethyl iodide and then hydrolysing the condensed product, ethyl 1-carboethoxy 1-ethyl 2-ethoxy methyl 3-cyano butyrate (9) However in this preparation dl-homo pilopic acid (10) had formed along with an isomeric acid. These two could be separated from each other with difficulty through the formation of their ethyl esters.

$$\begin{array}{l} CNCH_2\ CH.CH_2OC_2H_5 \\ \qquad\ | \\ \qquad CH(COOC_2H_5)_2 \end{array} \xrightarrow{C_2H_5I} \begin{array}{l} CNCH_2\ CH.CH_2OC_2H_5 \\ \qquad\quad | \\ C_2H_5.C(COOC_2H_5)_2 \end{array} \longrightarrow$$

(7) (9)

$$\begin{array}{l} C_2H_5\ CH \text{———} CH.CH_2\ COOH \\ \qquad | \qquad\qquad | \\ \qquad CO-O-CH_2 \end{array} \quad \text{and} \quad \begin{array}{l} CH_2 \text{———} CH.CH(C_2H_5)\ COOH \\ | \qquad\qquad\quad | \\ CO-O-CH_2 \end{array}$$

(10) (11)

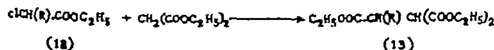
However when benzyl chloride instead of ethyl iodide was condensed with ethyl 1-carboethoxy 2-ethoxy methyl 3-cyano butyrate (7) two isomeric lactonic acids formed could not be separated from each other through the formation of their ethyl esters and also the yield was found to be extremely poor.

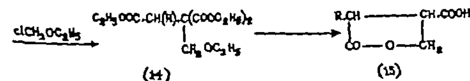
Due to the difficulties mentioned above, these lactonic acids were thus prepared by a process partly based on Kolbe's electrolytic method, using ...

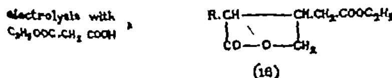
table mixtures of carboxylic acids. From the literature it has been found that practically all types of carboxylic acids, including half esters of acids upon electrolysis give corresponding Kolbe products. While the electrolysis of mixtures of acids give a mixture of products formed due to simple and cross coupling. Thus a mixture of two acids R.COOH and R COOH due to simple coupling will yield R R and R. R and due to cross coupling R.R It has been found that an increase in the amounts of any one of the acid leads to an increase in the amount of cross coupled product. It has also been found by earlier workers that even those acids which normally give little or no yield of Kolbe products when electrolysed alone, give cross coupled products easily with other acids in fair yields. Furthermore the mixtures of the products formed by the electrolysis are separable by simple procedures.

In the present investigation, with a view to standardise the electrolytic method an ester (yield 32 %) ethyl n-coproate, phenoxy ethyl benzene (yield 13 7%) Lauryl alcohol (yield 27 4%) Myristyl alcohol (yield 24 7 %) Cetyl alcohol (yield 13 7%) Tridecanol-2 (yield 20%) Pentadecanol 2 (yield 22 4) and Heptadecanol 2 (yield 13 2 %) had been prepared by cross coupling of suitable mixtures of acids.

Next, for the preparation of ethyl homo pilosinate (16, R=H) by electrolysis, first pilosinic acid (15 R=H) had been prepared by condensing diethyl malonate with ethyl monochloro acetate (12 R=H) and the resultant product, diethyl 1-carboethoxy succinate (13, R=H) was further condensed with mono chloro methyl ethyl ether Diethyl 1-carboethoxy 1-ethoxy methyl succinaet (14 R=H) thus prepared was then hydrolysed to pilosinic acid (15 R=H) Finally pilosinic acid was electrolysed with ethyl hydrogen malonate, when ethyl homo pilosinate in 32 56% yield was produced.







Similarly for the preparation of ethyl dl-homo pilopiate (16 R=C_2H_5) by electrolysis, ethyl a-bromo butyrate (12 R=C_2H_5) was condensed with diethyl malonate, when diethyl 1-carboethoxy 2-ethyl succinate (13, R_1=CH_3)

was obtained This was condensed with mono chloro methyl ethyl ether in order to prepare diethyl 1-carboethoxy 1-ethoxy methyl 2-ethyl succinate (14 R = C_2H_5) The latter on hydrolysis gave dl-pilopic acid (15, R = C_2H_5), which was cross coupled with ethyl hydrogen malonate, electrolytically also ethyl dl-homo pilopiate (16 R = C_2H_5) in 20% yield was obtained.

Thus electrolytic method was found to be quite convenient route for the preparation of various r-lactonic acids. However these acids were not utilised further for the preparation of jaborandi alkaloids because, simultaneously a more satisfactory and direct method for the purpose was developed, as described in sub-section C.

Sub-section C Synthesis of Jaborandi alkaloids

Preobrashenski and others had synthesised dl-pilocarpidine by converting d homo pilopyl chloride into diazomethyl d-homo pilopyl ketone, transforming the latter by the action of hydrochloric acid into d-homo pilopyl chloro methyl ketone. From this phthalimido-methyl d-homo pilopyl ketone was prepared, which upon hydrolysis gave amino methyl d-homo pilopyl ketone, the latter with potassium thiocyanate yielded 2-thiol 5-d-homo pilopyl ketone. The thiol upon oxidation of ferric chloride and subsequent methylation gave dl pilocarpidine and pilocarpine respectively Dey had pepared pilocarpine and isopilocarpine by converting the acid chloride of dl-homo pilopic acid with methyl zinc iodide to b-acetonyl a-ethyl r-butyro lactone and transforming the same to its benzylidine derivative by benzaldehyde. The ozonide of this benzylidine derivative on decomposition gave a glyoxal a-ethyl r-butyro lactone b-pyruvaldehyde. This glyoxal on treatment with ammonia and formaldehyde gave pilocarpidine and isopilocarpidine, which was converted into pilocarpine and isopilocarpine by methylation.

In order to prepare the jaborandi alkaloids in the present work, ethyl 3-phthalimido acetoacetate (23 R = H) was condensed with 1-ethoxy 3-cyano 2 propene (6) The resulting product on hydrolysis gave directly amino methyl homo pilosinyl ketone (19) which on treatment with an aqueous solution of potassium thiocyanate gave 2 thiol pilosinidine. The latter on oxidation and subsequent methylation gave the alkaloid pilosinine (22)

Ethyl 3-phthalimido acetoacetate (23 R = H) was first prepared by the method described by Gabriel, in which sodium derivative of diethyl malonate was condensed with phthalimido acetyl chloride, but the yield was found to be poor However when instead of sodium salt, ethoxy magnesium derivative of diethyl malonate was used, the desired product was obtained in 65% yield. An attempt to produce ethyl 1-alkyl 3-phthalimido acetoacetate (by condensing ethyl 3-phthalimido acetoacetate (23 R = H) with allyl halides using sodium ethoxide as condensing agent, could result only in the product of a deep red syrupy mass from which the desired product could not be crystallised

allised out. Ethyl 3-phthalimido acetoacetate (23 R=H) was then condensed with 1-ethoxy 3-cyano 2 propene (6) but the intermediate, ethyl 1-phthalimido aceto 2-ethoxy methyl 3-cyano butyrate (18) could not be crystallised out from the red coloured reaction product. However when this product was hydrolysed, extremely hygroscopic salt of amino methyl homo pilosinyl ketone (19) was produced. This ketone was treated with potassium thiocyanate when 2 thiol pilosinidine (20) was obtained. This upon oxidation and subsequent methylation gave pilosinine (22)

In an attempt to isolate the intermediate, ethyl 1 phthalimido aceto 2-ethoxy methyl 3-cyano butyrate (18) first ethyl 1-alkyl 3-phthalimido acetoacetate (23) were prepared in sufficient yield by condensing ethoxy magnesium derivative of diethyl alkyl malonate with phthalimido acetyl chloride in ether medium. Similarly when phthalimido acetyl chloride was condensed with ethoxy magnesium derivative of ethyl 1-carboethoxy 2-ethoxy methyl 3-cyano butyrate (17) and steam distilling the reaction product, ethyl 1-phthalimido aceto 2-ethoxy methyl 3-cyano buytrate (18) was obtained in good yield. The latter on hydrolysis gave amino methyl homo pilosinyl ketone (19) which was converted into pilosinine (21) as described above.

$CNCH_2.CH.CH_2OC_2H_5$ / $CH(COOC_2H_5)_2$ (7) → $CNCH_2.CH.CH_2OC_2H_5$ / $C_2H_5OMg.C(COOC_2H_5)_2$ (17) $C_6H_4(CO)_2N.CH_2Cl$ →

$CNCH_2.CH.CH_2OC_2H_5$ / $C_6H_4(CO)_2NCH_2.CO.C(COOC_2H_5)_2$ → $CNCH_2.CH.CH_2OC_2H_5$ / $C_6H_4(CO)_2N.CH_2.CO$ $CH.COOC_2H_5$ (18) →

CH_2—$CH.CH_2.CO.CH_2.NH_2$ / CO—O—CH_2 (19) —KCNS→ CH_2—$CH.CH_2.C$—NH / CO—O—CH_2 CH—N=$C.SH$ (20) →

CH_2—$CH.CH_2$ C—NH / CO—O—CH_2 CH—N=CH (21) → CH_2—CH CH_2 C—N—CH_3 / CO—O—CH_2 CH—N=CH (22)

In an another preparation, ethyl group was introduced in the methylene bridge of pilosinine. For this purpose ethyl 1-ethyl 3-phthalimido aceto-

acetate (23 $R=C_2H_5$) was condensed with 1-ethoxy 3-cyano 3-propene (6) in presence of sodium ethoxide and without isolating the intermediate compounds, the reaction mixture was hydrolysed and the amino ketone (24) obtained was converted into 2 thiol 6-ethyl pilosinidine (25) The latter upon oxidation and methylation as described above gave 6-ethyl pilosinine (27).

$$C_6H_4(CO)_2N.CH_2.CO.CH(R)COOC_2H_5 + CNCH{=}CH.CH_2OC_2H_5 \xrightarrow{NaOC_2H_5}$$

(23) (6)

$$\begin{array}{c} CNCH_2.CH.CH_2OC_2H_5 \\ | \\ C_6H_4(CO)_2N.CH_2.CO\ C(R)(COOC_2H_5) \end{array} \longrightarrow \begin{array}{l} CH_2 \text{---} CH.CH(R)\ CO.CH_2.NH_2 \\ | \qquad\qquad | \\ CO\text{---}O\text{---}CH_2 \end{array} \longrightarrow$$

(24)

$$\begin{array}{l} CH_2 \text{---} CH.CH(R)\ C \text{---} NH \\ | \qquad\qquad | \qquad\quad \| \qquad | \\ CO\text{---}O\text{---}CH_2 \quad CH\text{---}N\text{---}C.SH \end{array} \longrightarrow \begin{array}{l} CH_2 \text{---} CH.CH(R).C \text{---} NH \\ | \qquad\qquad | \qquad\quad \| \qquad | \\ CO\text{---}O\text{---}CH_2 \quad CH\text{---}N{=}CH \end{array}$$

(25) (26)

$$\longrightarrow \begin{array}{l} CH \text{---} CH.CH(R)\ C \text{---} N\text{---}CH \\ | \qquad\qquad | \qquad\quad \| \qquad | \\ CO\text{---}O\text{---}CH_2 \quad CH\text{---}N{=}CH \end{array}$$

(27)

Part III Separation of Alkaloids from Drugs

In view of the fact that alkaloids find uses both as therapeutic agents and as poisons, their importance in the field of medicines is self indicated. In India the number of medicinal plants and alkaloidal herbs grown is fairly high and it will be in the fitness of things under the present conditions to make attempt for separating these alkaloids from such plants and aquire self-sufficiency in this field.

The conventional methods are mostly emperical and make use of a good deal of solvents. Due to the tropical conditions prevailing in India, high costs are encountered, if such methods are used particularly during distillation and recovery from the drugs. In the present work it has been our intention to develop better methods which are quicker and at the same requirement for the solvents are minimum. We have utilized two methods for this purpose (A) Precipitation and (B) ion exchange.

(a) *Precipitation Methods*

From the results of North and Beal, Lamette et al and our own on the silicotungatates of pilocarpine it has been found that most of alkaloids are precipitated quantitatively as insoluble silicotungstates from the acidic solutions

Silicotungstic acid can thus be utilized for the separation of alkaloids from the acidic extracts of the plant materials, in which they easily come because of their basic properties. We have used this acid for the separation of alkaloids from plant materials and the results of the recovery of alkaloids given in table I indicates that this method can economically be used for their separation. Also in this method there is practically no loss of the silicotungstic acid and the alkaloids extracted need not be purified by the double shake method.

TABLE I

Name of drug	Assay results	Amt. of drug used in gms.	Amt. of alkaloid in drug calcd. on the basis of assay in gms.	Amt. of alkaloid extd. in gms.	% of alkaloid extd.
Tobacco	2 89 %	1000	28 9	28 9	100
Hyoscyamus niger	0 0611 %	5000	3 055	2 8247	92 46
Nux vomica	2 108 %	1000	21 08	19 8729	94 27
Cinchona	5 382 %	500	26 91	26 1023	96 99
Jaborandi	0 6105 %	2000	12 40	11 9760	98 16
Rauwolfia Serpentina	1 01 %	2000	20 2	18 5212	90 79

(b) *Ion Exchange method*

The use of absorption technique is comparatively recent, especially ion exchange is best suited for the purpose, as the alkaloids form basic cations due to their basic nitrogen. Ion exchange process have been used in other countries for this purpose, the details are patented and guarded secrets. We have utilized certain grades of sulphonated coals (including one prepared by us) zeo carbs 215, 315 and Na as exchangers.

The property that the exchangers which swells more in water differentiates less between cations in the strength of absorption, is in accordance with the observed behaviour of zeo carb 215. This exchanger also has greater ion exchange capacity for alkaloids and therefore it has been selected for the large scale separation of alkaloids from the drugs.

The process of absorption has mostly been found in cases of all exchangers to follow logrithmic type of distribution law. In order to ascertain this finding the values of k and p used in the equation $x = k \log cp$ (where x is the amount of alkaloid absorbed per gram of the exchanger, c is the concentration of the solution employed, p and k are constants) have been found for zeo carb 215 for deferent alkaloids with the help of a graph.

Effect of concentration and time on the exchanging power of the exchangers has also been studied with the help of individual alkaloids. From the curves drawn between time and amount of alkaloids absorbed in millimoles per gram of the exchanger it is clear that the process of exchange is of long duration and all the alkaloid cannot be sepatated by a given quantity of the exchanger even after weeks (Saunders and Srivastava) The steep rise in the curves indicates that the exchange is fast in the beginning but sloved down with increase in time. These curves also reveal that the amount of the base absorbed is directly proportional to the concentration of the solution used. Further these graphs have been used as reference graphs for determining the amount of the alkaloids absorbed at different times with in the concentrations of the solutions used in the present investigation.

If the rate of exchange per gram of the exchanger per unit time is plotted against the corresponding time, the curves for different concentrations reveal two significant points. Firstly the rate of exchange per gram of exchanger per unit time is more or less independent of concentration. This result is drawn from the observation that such curves are in most cases, coincedent with each other even when concentration differ widely Secondly the steep fall in the curves indicates that the rate of exchange is fast in the first few hours and after which it becomes almost constant. Thus it is uneconomical to keep the solution of the alkaloid in contact with the exchanger for long duration with a view to remove all the alkaloid from the plant extract. These curves have been helpful in finding the economical time during which the exchanger should be kept in contact with the plant extract and there after the exchanger column must be changed. This time factor has been approximately determined by drawing a line parallel to the time axis through the point where the tangent drawn at the ends of the curves meet each other The point where this line meet the curve give the economical time factor This time factor for all the four exchangers have been determined for different alkaloids.

In order to demonstrate the applicability of these observations, we have used zeo carb 215 for the separation of alkaloids from different plant extracts. The results are given in table given below

TABLE 2

drug	Amt. of drug used in gms.	Amt. of zeo carb 215 used in grams	Amt. of alkaloid absorbed in gms.	Amt. of alkaloid absorbed as calcd in gms.	Amt. of alkaloid eluted in gms.	% of the alkaloid eluted
Tobacco	1750	200	28.9302	33.91	28.2804	97
Ephedra	6000	100	49 7526	51 720	49.6462	99.3
Nux vomica	4000	800	62.38	66.40	61.0717	97.4
Hyoscyamus niger	5000	40	all	5.5017	2.917	100.0
Jaborandi	500	150	17 15850		16.9217	95.62
Rauwolfia Serpentina	5000	1000	30 4		28.9120	95.95

MORPHOLOGICAL STUDIES ON EUCHLAENA MEXICANA SCHRAD

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Euchlaena mexicana Schrad., Maydeae, Gramineae, is commonly known as teosinte. It grows as weed and is also cultivated in the warmest parts of North America extending from North Central Mexico to Hundras (Weatherwax, 1935)

Euchlaena has been described several times in connection with the origin of maize with which it hybridizes freely Ever since Harshberger produced fertile hybrids between maize and teosinte in 1896, many authors have described the cytology and inheritance of these hybrids involving various races of teosinte. This work has been well reviewed among others by Mangelsdorf (1947) and Randolph (1955 1959) The role that teosinte might have played in the origin of maize has been variously interpreted Ascherson (1880) and Longley (1941) have suggested that maize arose as a result of mutation from *Euchlaena*, while Collins (1912) and Harshberger (1893) state that maize is a hybrid between teosinte and some other grasses of Andropogoneae.

Beadle (1939) states that annual teosinte is similar to maize in having 10 pairs of chromosomes also that there occurs normal crossing over between the two He further states that other cytological details also show that in germplasm architecture both maize and teosinte are remarkably alike and placing them in separate genera is perhaps no longer justified Reeves & Mangelsdorf (1942) treat *Zea* and *Euchlaena* as two species of the same genus and have named latter as *Zea mexicana* They write, In gross morphology neither genus is known to have a character that is lacking in other"

Recently Singh & Paliwal (1960) have described a luxuriant form of teosinte called by them as B R. C teosinte. Koul & Paliwal (1961) have described bulbil formation and cytological details in maize X B R. C teosinte hybrids. On the basis of these studies they conclude that B R. C teosinte resembles Guatemalan teosintes. The present study is based on the material collected from this teosinte growing in Botanic Gardens of B R. College, Agra It aims at accruing the meagre information available regarding the embryogeny ond floral morphology of this plant.

The present work was started by Dr Bahadur Singh, who studied embryological stages. The material as well as the slides were then passed on by him to Sri Sahi who further extended it and submitted the work in the form of M. Sc thesis. Since he did not find time to extend it further Dr Bahadur Singh handed over the material to Sri A. K. Koul. The present paper is thus the result of the findings of these authors.

Synonyms, *Euchlaena luxurians* dur & *Reana luxurians* dur (Arber 1934) *Zea mexicana* (Reeves & Mangelsdorf 1942)

Review of Previous Work

Cooper (1938) investigated the embryo sac development, its fertilization and early stages of endosperm and embryo in *Zea mays*, *Euchlaena mexicana* and their F1 hybrids. Koul (1959) has described the behaviour of antipodals during caryopsis formation in *Euchlaena mexicana*. Miller (1919) has investigated the development of pistillate spikelet of *Zea mays*. Bonnet (1940) has worked out the development of pistillate and staminate spikelets which he thinks arise acropetally as lateral projections. Laubengayer (1949) has described the vascular anatomy of 8-rowed ear and tassel of maize. Reeves (1928) has reported partition wall formation in pollen mother cells of *Zea mays*. Poindexter (1903) states that pollen grains of *Zea* are shed at 3-celled stage.

True (1893), Randolph (1936) and Johann (1942) while studying the developmental morphology of caryopsis in maize, found that, because of early degeneration of integuments the real seed coat is wanting. Gordon (1922), Weatherwax (1926, 1930) and Sass (1946) report the persistance of antipodal tissue in young seeds of *Zea*. Weatherwax (1930) has made a comparative study of the endosperm of *Coix* and *Zea* in both of which he has observed ... of the endosperm. Weatherwax (1920) has discussed the position of scutellum and homology of coleoptile in maize. He concludes that according to evidence derived from the structure and development of maize embryo the coleoptile is the homologue of a foliage leaf while the cotyledon is a lateral organ. Avery (1928, 1930) while making a comparative anatomical and morphological study of the seedlings of *Zea* and other grasses concludes, that scutellum in maize, wheat and oats is the cotyledon.

Fraquharson (1954) has worked out the complete embryogeny of diploid and tetraploid races of *Tripsacum dactyloides*. In a later publication (1955) she has reported the frequent occurrence of apomixis and polyembryony in *T. dactyloides*.

Material and Methods

Young spikes, both male and female were fixed on the spot as such in formalin-acetic-alcohol while for older female spikelets the glumes were removed to facilitate proper penetration of the fluid. Tertiary butyl alcohol as well as alcohol-xylol series were used for dehydration. Sections were cut of the order of 8-15 μ and were stained in safranin fast green and Heidenhain's iron alum haematoxylin, both of which gave good results. Paliwal's technique (1955) was used for micro-dissection of embryo sacs.

Observations

Inflorescence and flower. *Euchlaena mexicana* is a monoecious plant with stamens and pistils borne separately on tassel and cob respectively. The tassel arises terminally and bears a variable number of spirally arranged lateral branches. The central spike of the type seen in the tassel of maize is lacking in *Euchlaena*.

Spikelets arise in pairs, of which one is sessile and the other pedicelled (Fig 1) Each spikelet bears at its base a pair of opposite glumes. Each staminate flower possesses an outer bract, the lemma and an inner bract, the palea. The lemma is more or less ovate, convexo-concave and glabrous. The palet, inserted opposite the lemma, is thin and membranous, nearly flat with inturned margins. Each flower bears two lodicules which at the time of anthesis of the flower swell to two or three times their original size. Of the three stamens that each flower bears one is situated dorsally opposite the palet while two others are arranged on the side of palet (Fig 2). The bilobed and versatile anthers may either be brown or pink in colour. Besides, each male flower bears a rudimentary gynoecium.

The pistillate inflorescence arises laterally and is commonly known as cob (Fig 3). It is a spike of spikelets. Spikelets arise singly and acropetally on a zigzag rachis. Each spikelet is enveloped in two opposite glumes and consists of two flowers of which the upper is fertile and the lower sterile (Fig 4). The gynoecium is monocarpellary, hypogynous and unilocular bearing a long terminal, hairy silk of a disputed morphology*. The lower sterile flower of the spikelet resembles the fertile flower in all respects except that it lacks a gynoecium. On maturity the two glumes become hard and dark brown and completely enclose the mature seed.

Anther The young anther of *Euchlaena* is somewhat quadrangular in cross section with four distinct lobes. The earliest stage available for study was the one where the archesporium had already divided and produced parietal layer from outside and the sporogenous tissue from inside (Fig 5). Later the parietal layer divides periclinally cutting off endothecium and an inner layer (Fig 6). Cells of the latter undergo a periclinal division to produce a single layered middle layer from outside and tapetum towards inside (Fig 7). The parietal tissue in a mature anther consists of epidermis, a single layered endothecium, a middle layer and a single layered tapetum.

The epidermal layer is composed of a single row of rectangular parenchymatous cells with outer walls somewhat convex (Fig 7). These cells elongate tangentially to keep pace with the growing anther. The cells show signs of degeneration at about the time the microspores are separated.

In early stages the endothecium is formed of ordinary parenchymatous cells which increase in size as the anther matures (Fig 7). Between the endothecium and the tapetum, there is a single row of thin walled, elongated cells that constitute the middle layer. With the beginning of meiotic division in microspore mother cells, the cells of the middle layer start getting crushed (Fig 8) and by the time pollen grains are formed they degenerate completely.

The tapetum is a single layered glandular tissue composed of conspicuous, isodiametric cells which contain abundant cytoplasm and prominent nuclei.

*While some morphologists call the silk as the style others think it to be the stigma.

At the time the pollen mother cells are undergoing pachytene the tapetal cells have already passed from the uninucleate to the binucleate condition (Fig. 8). Similar observations have been made by Nirodi (1955) in *Coix*, *Polytoca* and *Chionachne* and Koul & Paliwal (1961) in a 32-chromosome *Coix*.

Microsporogenesis The primary sporogenous cell divides twice or thrice resulting in the formation of 6–8 sporogenous cells in a cross section of the pollen sac (Fig 7) The microspore mother cells that are arranged in two rows in the L S of the anther (Fig 9) have dense cytoplasm and prominent nucleus at early stages Later on preparatory to their entry into prophase of meiosis they grow considerably become rounded up (Fig 10) and get separated from one another

The division of microspore mother cells is synchronous. The primary spindle is formed in the middle of a mother cell (Fig 11) The cell plate laid down at the equatorial region divides the mother cell into two dyad cells (Fig 12) The second division results in the formation of isobilateral microspore tetrads (Figs. 13 14 & 15) A microspore appears somewhat triangular and it always has its tapering end facing the centre of the tetrad (Fig. 15). This shape, however is lost before long when each microspore acquires an oval or spherical outline with a centrally placed nucleus that remains embedded in homogeneous cytoplasm (Fig 16)

The young uninucleate pollen grain acquires a thick exine. Subsequently its nucleus is pushed towards the wall where it undergoes a mitotic division producing a large vegetative and a small generative cell The latter undergoes a division only after reaching the centre of the pollen grain (Fig 17) producing two somewhat rod shaped sperm cells, which take quite a bright stain with aceto-carmine (Fig 18)

Ovule The ovular primordium in *Euchlaena mexicana* is a dome shaped protuberance consisting of homogeneous, parenchymatous cells at the base of the ovarian cavity A single hypodermal cell at the apical region of this dome is distinguishable from the rest of the tissue by virtue of its bigger size denser cytoplasm and conspicuous nucleus (Fig 19) It is the female archesporium In a few cases two to three archesporial cells are seen lying side by side (Fig 20). By the time the archesporial cell is established, integuments arise laterally at the base of the nucellus. The inner appears first and is shortly followed by the outer The ovule at this stage starts bending by degrees towards the axis of the spike (Fig 21) and at maturity it acquires a form intermediate to an amphi- and anatropous ovule.

The inner integument at maturity remains two layered and covers the ovule in entirety except at the tip where it forms a narrow micropyle The outer integument is 4 5 layered at the base but only two layered elsewhere It covers only three fourths of the ovule. By the time the megaspore tetrads are

formed the outer integument starts wedging in the form of a hump into the stylar canal (Fig 21) Throughout the course of these events the ovule grows considerably in size

Megasporogenesis The hypodermal archesporial cell which becomes deep seated due to the periclinal division of the cells of the epidermis, enlarges and directly functions as the megaspore mother cell (Fig 22) A linear megaspore t trad is formed as a result of usual meiotic division of the mother cell (Fig 23) In a few cases two linear tetrads of megaspores were suspected growing side by side. Of four megaspores, th three micropylar ones degenerate while only the chalazal megaspore survives (Fig 24) Degeneration starts from the micropylar end and remains of the degenerating megaspores can be made out up to the two or even four-nucleate embryo sac stages.

Development f the female gametophyt The functional megaspore enlarges considerably as its nucleus undergoes three successive divisions leading to th for mation of two- four and eight-nucleate embryo sacs (Figs. 25 26 & 27) The development of the embryo sac conforms to *Polygonum* type (Maheshwari, 1950)

The organized female gametophyte shows a 3-celled egg apparatus, 2 polar nuclei and three antipodal cells (Fig 28) All th three cells of egg apparatus, which consists of an egg and two synergids, are somewhat pear shaped with the narrower end extending towards the micropyle. Nuclei in the two synergids lie almost in centre, immediately above a basal vacuole. In th egg cell, vacuole occupies the micropylar end, while its nucleus remains embedded in the dense cytoplasm. With maturation however a number of vacuoles appear and the egg is found to have grown down appreciably into the embryo sac.

The three antipodal cells, soon after they are formed, divide mitotically giving rise to a mass of several multinucleate cells (Figs 29 & 30) Besides an increase in number the antipodal cells also show a considerable enlargement in size (Fig. 30) The behaviour of antipodal complex in *Euchlaena mexicana* has been reported already in an earlier communication (Koul 1959) The antipodal cells show maximum growth at mature embryo sac or at free nucleate endosperm stage. As reported in maize by Randolph (1936) Weatherwax (1926) and Cooper (1938) the antipodals in teosinte also are traceable even up to young seed stage (Fig 31) The behaviour of antipodals is clearer in microdissections than that possible with serial sections.

Polyembryony Though polyembryony and apomixis is of frequent occur rence in Andropogoneae, in Maydeae th reports are so far restricted only to *Tripsacum* (Farquharson, 1955) During the course of present investigation W have come across a few twin embryo sacs closely appressed with each other (Fig 32) Each of these embryo sacs has a distinct egg apparatus, two polar nuclei and the antipodal complex. Because the frequency of twin embryo sacs

is very small their mode of origin and ultimate fate could not be worked out. The occurrence of multiple archesporia and two megaspore tetrads, however, offers a tentative suggestion that these twin embryo sacs may have arisen from two independent megaspore mother cells.

While transplanting seedlings of *Euchlaena*, a few twin seedlings were observed (Fig 33) On removing the glumes carefully it was confirmed that the seedlings arose from a single seed enclosed in hard glumes suggesting the survival of twin embryo sacs to form viable embryos.

Pollination and fertilization *Euchlaena* like *Zea* is also a cross pollinated plant. As the flowers mature anthers hang out and dehisce (Fig. 34) The pollen is shed little by little as the versatile anthers dangle at the end of the filaments. Wind and gravity constitute the chief agencies of pollination. Occasionally insects and flies also prove helpful. The silks are receptive right from the time of their emergence. Pollen can germinate on any part of the silk and consequently some morphologists take silk to be stigmatic in nature

Cooper (1938) reports that fertilization takes place between 15-20 hours after pollination. Fertilization is porogamous, resulting in the formation of a zygote and a primary endosperm nucleus (Fig 29)

Endosperm The division of the primary endosperm nucleus takes place much before the division of the zygote. A quick succession of nuclear divisions produces a large number of endosperm nuclei which immediately after their formation take up a peripheral position in the embryo sac. These nuclei remain in connection with one another through intersecting, twisting and overlapping cytoplasmic strands (Fig 35)

Wall formation commences only after a fairly large number of endosperm nuclei have been produced. It starts at the periphery and extends towards the centre till the whole of the endosperm becomes cellular (Fig 36) Cells of the endosperm in the neighbourhood of the embryo undergo rapid and repeated divisions in contrast to those situated in the antipodal region. Due to rapid enlargement of endosperm in all directions the thin walled parenchymatous cells of the nucellus are by and by ruptured and the endosperm comes in contact with integuments.

The endosperm in advanced stages of development shows a great deal of lobing laterally and distally (Fig 37) Lobing of endosperm has also been observed by Sass (1946) in the distal end of endosperm in Argentine Wx maize Sunila lobing has also been reported in other strains of maize as (See Sass, 1946) The lobing of endosperm in Gramineae is attributed by Sass (1946) to irregular meristematic activity of the peripheral cells of the endosperm Narayanaswami (1955) on the other hand thinks that lobing of endosperm takes place by the involution of the surface of the kernel. Endosperm cells

towards the placenta are greatly elongated looking much unlike the neighbouring cells (Fig 38) Weatherwax (1930) has come across similar cells in the endosperm of *Zea* and *Coix* to which he ascribes a temporary vascular function

The present study shows that the cells of the outermost layer of nearly mature endosperm of *Euchlaena* are meristematic they undergo a number of periclinal divisions producing several layers of cells which are arranged one above the other (Fig 39) The aleurone layer which constitutes the outermost layer of endosperm consists of well defined cubical cells enveloping the endosperm. The first evidence of the storage of starch in the endosperm appears at the end farthest from the embryo In course of time all cells, with the exception of the cells of the aleurone layer and a few others next to scutellum, become heavily packed with starch (Fig 40) The amount of starch in cells of the chalazal end is considerably more decreasing gradually towards the micropylar end. This is a characteristic pattern of starch deposition comparable to that observed in Flint and Popcorn (Sass, 1946 1955)

The cells towards the aleurone layer are narrower elongated and mostly rectangular The cell size, however increases towards centre where they are quite big and polygonal in outline A variation in size and number is also displayed by starch grains, which are small in size and number in peripheral endosperm cells gradually increasing in size and number towards the centre. The number of starch grains per cell as seen in transverse sections of endosperm varies from 20-40 in smaller peripheral cells and 40-60 in central bigger cells. The starch grains are simple and range in outline, from oval to round, the latter type being more frequent.

The behaviour of nuclei of endosperm cells is quite interesting They differ in relative chromaticity size, shape and vacuolation from outer to central region of endosperm. Nuclei of peripheral cells are round, small and non vacuolated having a deep staining capacity and lying towards the centre of the cell. The size of nuclei, as we proceed towards centre increases while their chromaticity decreases. Each nucleus of the central endosperm cells includes a single nucleolus, occasionally however nuclei with two nucleoli may also be met with. Nuclei display variable shapes, the most common ones being oval elongated, triangular and amoeboid.

Embryo The zygote undergoes a period of rest during which its cytoplasm becomes homogeneous. There is considerable elongation of the zygote before it undergoes division Its first division is transverse to the long axis of the embryo sac. Cooper (1938) has described that, by the time 25-30 endosperm nuclei are formed, the embryo attains only a four-celled stage. The present investigation shows that globula embryo is formed by the time most of the endosperm has become cellular (Fig 36) The cells of embryonal base divide forming a suspensor which is 6-7 cells broad and about 35 cells long (Fig 42) It pushes the club shaped embryo deep into the endosperm.

In later stages, the embryonal mass shows three prominent lobes (Fig. 42) The posterior one of which gives rise to the proximal part of scutellum, the oblique distal lobe produces the distal extension of the scutellum while the anterior lobe forms the plumule, (Figs. 42 & 43) The development of embryo closely resembles that of maize (Sass, 1955) A rough club shaped embryo develops a lateral depression wherein arises the plumular primordium (Fig. 43) As the latter grows it is surrounded by an annular outgrowth the nature of which could not be ascertained at this stage. In a mature embryo, however the plumule is found to be enclosed in a coleoptile which is again situated in the tube-like outgrowth of the scutellum From the stem tip arise the initials of first two leaves while those of the third, fourth and fifth leaves arise later on (Fig 44) The leaf blades expand and edges of the older leaves overlap those of the younger ones. The coleorhiza and root cap differentiate from the radicle primordium. The radicle primordium and the coleorhiza is clearly seen as a dome shaped tip projecting out of th ring of the scutellum base. As the parts of the embryo start differentiating the suspensor disappears totally

The form of scutellum is the most interesting feature found in the present investigation In *E. mexicana* it forms a hollow cylinder completely surrounding the plumule as well as the radicle (Figs 44 45 46 & 47) The wall of the scutellar cylinder is not of uniform thickness. The scutellum fuses with the mesocotyl. The upper part of the scutellum, above the point of insertion of the plumule elongates and is somewhat reflexed This forms a somewhat concavo-convex plate whose concave side is appressed to the endosperm. Fig 48 shows the orientation of mature embryo in the caryopsis of *Euchlaena*.

Th scutellum is composed of densely packed, oval or hexagonal cells with very small or no intercellular spaces. The epidermal cells of the scutellum on the side opposite to that of endosperm are slightly elongated and feebly cytoplasmic while those on the side of the endosperm are smaller radially elongated with dense cytoplasm suggesting them to be suctorial in function. Similar views have also been put forth by Sargant & Robertson (1905) while working on the endosperm of maize The vascular system of the scutellum is represented by a broad strand of elongated thin walled cells running along its median line from which arise fine branches throughout its length. A vascular procambial strand supplies the lower part of the scutellum. From the mesocotyl arise vascular supplies to the plumule and radicle.

The mesocotyl, an extremely short transitional region between root and stem, has been interpreted as (a) fusion of hypocotyl and stalk of the scutellum (Sargant & Arber 1915) or (b) the first internode (Avery 1930) The plumule consists of a growing point surrounded by 3-5 rudimentary leaves. The first leaf which arises opposite to the main body of the scutellum serves as coleoptile the protective organ The plumule mesocotyl and radicle are in a straight line from distal to the proximal end of an embryo. The tip of the pro-

mary root is enveloped by a root cap which forms an inverted pyramid The coleorhiza completely encloses both the primary root and its cap (Fig 44)

Seed and Fruit The grain in *E mexicana* remains enclosed in an indurated husk, which is the product of the glume and rachis (Fig 49) The fruit is small, more or less conical in shape, dark brown in colour and hard in texture having a smooth surface (Fig 50) It is a simple, dry indehiscent, one seeded caryopsis The seed is adherent to the wall of the ovary and is not separable from it Lemma and palea are persistent and, in a mature caryopsis, intimately cover th fruit. The grain consists of endosperm embryo nucellus and fruit coat.

Whole of the nucellar tissue, except the nucellar epidermis, collapses during the vigorous development of the endosperm. Maturation of caryopsis results in a progressive degeneration of the outer integument (Fig 51) The only remains of the inner integument are represented by a small layer of cuticle recognizable towards the placental region the rest of it is also completely lost like the outer integument (Fig 52) It is interesting to find that *Euchlaena* grain does not possess any seed-coat and is, therfore, naked It is enveloped by the pericarp directly The pericarp develops from the outer epidermis and a few degenerated hypodermal layers of the ovary wall.

DISCUSSION

Euchlaena and *Zea* display a close morphological and cytological resemblance. The chromosome number as well as the number of their arms is exactly alike in both genera (Beadle, 1939) Fertile hybrids between the two occur in nature. Beadle (1939) reports that crossing over between maize and teosinte chromosomes is necessarily normal except for a short segment of chromosom 9 On the basis of these remarkable similarities of germplasm architecture Beadle (1939) pleads that the assignment of generic rank to *Euchlaena mexicana* as separate from *Zea mays* is perhaps not justified.

Reeves & Mangelsdorf (1942) have gone further ahead, and made a taxonomic change in the tribe Maydeae making *Euchlaena* and *Zea* congeneric and naming latter as *Zea mexicana*. They write, "In gross morphology neither genus is known to have a character that is lacking in the other Distichous ears, originally thought to be absent in *Zea* have been reported in this genus by Tavacar (1935) and Langham (1940) Paired pistillate spikelets have been found by Reeves & Mangelsdorf (unpublished) to be of fairly regular occurrence in a form of *Euchlaena*. The four main characters used to separate *Euchlaena* from *Zea* are (a) Disarticulating rachis, (b) Distichous spike (c) Solitary pistillate spikelets and (d) Covered grains. Reeves & Mangelsdorf (1942) however claim that In respect to all four characters, intergrading forms between *Zea* and *Euchlaena* are known Also Decandole has emphasized the error of classifying domesticated plants botanically on the basis of characters for which they are cultivated"

Similar views have also been expressed by Ascherson (1875) who described *Zea* as stunted type of *Euchlaena*. East (1939) stated that two groups are simply diverse types of the same polymorphic aggregation, although they may be called species (not genera) if one so desires. Langham (1938) has also expressed a similar view Koul & Paliwal (1961) also support this opinion.

Cooper (1938) was the first to point out the closeness of the two genera *Zea* and *Euchlaena* embryologically The origin of wall layers of an anther from the parietal cells is exactly similar in both forms. Meiotic divisions leading to the formation of microspores is normal the pollen grains in each case have a smooth exine, are monoporate, acolpate and are shed at the 3-celled stage.

Ovule in *Euchlaena* as also in *Zea*, is amphi-anatropous and bitegmic with the outer integument in each case covering only three-fourths of the ovule and forming a hump which penetrates the stylar canal. Primary archesporial cell in *Euchlaena* functions directly without division Cooper (1938) has come across a similar case in *Zea* Weatherwax, however described a periclinal division of the archesporium in Corn forming a parietal and the megaspore mother cell. Though the usual number of spore mother cells is one but occasionally two megaspore mother cells were observed during the present study Meiotic divisions of the megaspore mother cell result in the formation of linear tetrads of megaspores of which the micropylar three degenerate and only the chalazal one survives. Miller (1919) reports all four megaspores to participate in the formation of embryo sac in corn. Cooper (1938) reports the survival of only one megaspore in *Zea* as well as *Euchlaena*. During the present investigation we came across a few ovules with two tetrads lying side by side. Embryo sac development conforms to *Polygonum* type. The three antipodals divide immediately after they are formed producing a mass of 20-30 cells which are multinucleate and show an increased size. These persist even in young seeds. Antipodals behave similarly in corn as well (Weatherwax, 1926)

In *Euchlaena* we came across twin embryo sacs lying quite apposed to each other Such reports in the tribe Maydeae are hitherto confined only to *Tripsacum*. Emergence of twin seedlings from a single seed suggests the occurrence of Polyembryony probably a case of pseudopolyembryony

Endosperm development in *Euchlaena* is free nuclear This as well as the form of cells and the laying of starch grains is similar to *Zea*

Embryo development appears to be essentially similar to *Zea* in details. The tubular form of the scutellum in *Euchlaena* appears to be the only difference

The findings embodied in the present study show that the two plants are embryologically alike Thus embryological findings also lend support to the view already expressed by Reeves & Mangelsdorf (1942) that *Euchlaena* should be made congeneric with *Zea* and named as *Zea mexicana* Reeves & Mangels-

dorf (1942) write, Since the name *Zea* L was proposed prior to *Euchlaena* Schrad the International rules require that it be retained"

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References

1 Ascherson P 1875 Ueber *Euchlaena mexicana* Schrad. *Bot. Verein Prov. Brandenburg, Verhandl*, 17: 76-80

2. Ascherson, P 1880 : Bemerkungen über astigen Maiskolben. *Bot. Ver Prov. Bran.* 21 133-138

3. Avery (J) G S 1928 Coleoptile of *Zea mays* & other grasses. *Bot. Gaz.* 86 107-110

4. Avery (J) G S. 1930 Comparative anatomy and morphology of embryos & seedlings of maize, oats and wheats. *Bot. Gaz.* 89 1 39

5 Beadle, G W 1939 : Teosinte & the origin of maize. *Jour Heredity* 30 245-247

6. Bonnett O T 1940 : The development of staminate & pistillate inflorescences of sweet corn. *Jour Agri Res* 60: 25-57

7 Collins, G N 1912 The origin of maize. *Jour Washington (D C) Acad. Sc.* 2 520-530.

8. Cooper, D. C. 1938 Macrosporogenesis & embryo sac development in *Euchlaena mexicana* & *Zea mays Jour Agri. Res.* 55 539-551

9 East E. M. 1939 A chronicle of the tribe of corn. *Pop Sci Monthly* 82 225-236.

10. Farquharson, L. I 1954 : Apomixis, polyembryony and related problems in *Tripsacum* (Thesis) *Univ. Microfilms. Ann Arbor Mich.*

11 Farquharson L. I. 1955 Apomixis & polyembryony in *Tripsacum dactyloides. Amer Jour Bot.* 42: 737 745

12. Gordon, M. 1922 : The development of endosperm in cereals. *Proc. Roy. Soc. Victoria* 34: 105-116.

13 Harshberger, J W 1893 Maize. A botanical and economic study Contr *Bot. Lab. Univ. Pennsylvania* 1 75-202.

14 Harshberger, J W 1896 Fertile crosses of teosinte and maize. *Gdn. & Forest* 9 522 523.

15 Johann, H. 1942 Origin of the suberized and semipermeable memberane in the caryopsis of maize. *Jour Agri. Res* 64 275-282.

16. Koul, A. K. 1959 Antipodals during the development of caryopsis in *Euchlaena mexicana* Schrad. *Agra Univ J or Res* (Sci.) 8 31 34

17 Koul, A. K. & Paliwal, R L. 1961 Further studies on B. R. C. teosinte X maize hybrids. & Paliwal, *Agra Univ. Jour Res.* (Sci) 10 (2) 79-97

18. Koul, A. K. & Paliwal, R. L. 1961 A new species of *Coix* with 32 chromosomes. Cytologia (in press).

19 Langham, D G 1938 : The inheritance of intergeneric differences in *Zea-Euchlaena* hybrids. *Abstract in Records Genetics Soc. Amer.* 7 78.

20. LANGHAM D G 1940 The inheritance of intergeneric differences in Zea-Euchlaena hybrids. *Genetics* 25 88-107

21 LAUBENGAYER, R A. 1949 : Vascular anatomy of 8 rowed ear and tassel of golden bantam sweet corn. *Amer J of Bot* 36 236-244

22 LONGLEY A. E. 1941 : Chromosome morphology in maize & its relatives. Bot. Rev. 8 : 399-41..

23 MAHESHWARI P 1950 : An Introduction to embryology of angiosperms. Mc Graw Hill Publication.

24 MANGELSDORF P C. 194 : The origin and evolution of maize. *Advances in Genetics* 1 XI 207 Academic Press Inc. New York.

25. MILLER, E. 1919 : Development of pistillate spikelet and fertilization in Zea mays L. Jour *Agri. Res.* 18 255—266.

26. NARAYANASWAMI, S. 1955 : The structure & development of the caryopsis in some Indian millets- IV *Echinochloa frumentacea* *Phytomorphology* 5 161 171

27 NIRODI, N 1955 : Studies on Asiatic relatives of maize. *Ann. Missouri Bot. Garden* 42 103-132.

28. PALIWAL, R. L. 1953 : A new technique for the microdissections of embryo sacs of the Gramineae. *Phytomorphology* 3 118-121

29 POINTDEXTER, C. C. 1903 : The development of the spikelet and grain of corn. Ohio *Naturalist* 4 3-9

30 RANDOLPH, L. F 1936 Developmental morphology of caryopsis in maize *Jour Agri. Res* 53 881 916.

31 RANDOLPH, L. F 1955 History & origin of corn. II Cytogenetic aspects of the origin & evolutionary history of corn *I Corn & Corn Improvement.* Academic Press New York. 16-61

32 RANDOLPH L. F 1959 : The origin of maize. *Ind. Jour Genet & Pl. Breeding* 19 1-12

33 REEVES, R G 1928 Partition wall formation in the pollen mother cells of Zea mays Amer *Jour Bot.* 15 114-122

34 REEVES, R. G & Mangelsdorf, P C. 1942 A proposed taxonomic change in the tribe Maydeae (family Gramineae) *Amer Jour Bot.* 29 : 815-817

35. SARGANT E. & Robertson, A. 1905 : The anatomy of the scutellum of Zea mays. Ann. Bot 19 115-125

36. SARGANT E. & Arber A. 1915 : Comparative morphology of embryo and seedlings in the Gramineae *Ann. Bot.* 29 161 222.

37 SASS, J E. 1946 The development of endosperm and antipodal tissue in Argentine waxy maize. *Amer Jour Bot.* 33 791 795.

38. SASS, J E. 1955 Vegetative morphology of maize *In Corn & Corn Improvement.* Acad. Press. Inc. New York. 63-87

39 SINGH, B. & Paliwal, R L. 1960 Studies on luxuriant form of teosinte & its hybrids with maize. *Agra Univ. Jour Res* (Sci) 9 137-146.

40. TAVACAR, A. 1935 Beitrage zur Vererbung der Kornreihenanzahl & Maisfor... *tsche F Zucht., Pflanzenz.* 20 364-376.

41 TRUE, R H. 1893 On the development of caryopsis. *Bot. Gaz.* 18: 212-226.

42. WEATHERWAX, P 1920 Position of scutellum and homology of coleoptile in maize. Bot *Gaz.* 69 179-182.

43 WEATHERWAX, P 1926 Persistance of antipodal tissue in the development of seed in maize *Bull Torrey Bot. Club* 53: 381 384

44 WEATHERWAX, P 1930 The endosperm of *Zea* & *Coix*. *Amer Jour Bot* 17 371 380

45. WEATHERWAX, P 1955 Structure & development of reproductive organs. *J Corn & Corn Improvement* Academic Press In New York.

Explanation of Figures

Explanation of Plate I

Figs 1-4 Diagrammatic sketches of the infloresence and flower

Fig 1 A pair of male spikelets, of which one is sessile and the other pedicelled.

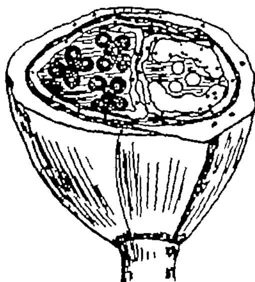
Fig 2. A male spikelet, cut half way transversely to show the arrangement of floral parts.

Fig 3 Pistillate inflorescence with partially opened spathe to show the arrangement of female spikelets.

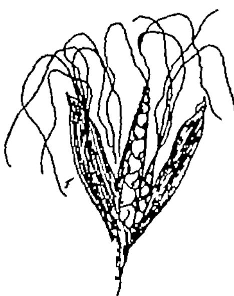
Fig 4 L. S of the female inflorescence showing the floral organs.

PLATE I









Explanation of Plate II

Figs. 5-18 Various stages of the development of anther and the male gametophyte

Fig 5 An anther lobe in T S. showing a parietal layer and two sporogenous cells X 1000

Fig 6 An anther lobe in T S showing from outside epidermis, endothecium an inner layer and a few sporogenous cells X 1000.

Fig 7 An anther lobe in T S showing from outside epidermis, endothecium, a middle layer and a single layered tapetum enclosing a mass of sporogenous cells X 900

Fig 8 A part of the anther in T S showing the compressed cells of the middle layer Note the binucleate condition of most of the tapetal cells X 900

Fig 9 A part of the anther in L. S showing sporogenous cells that are arranged in two rows.

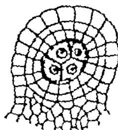
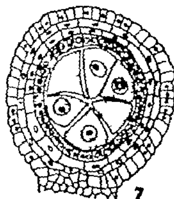
Figs. 10 11 12, 13 14 & 15 Microspore mother cells at various stages of meiosis, during the formation of male gametophyte X 2000

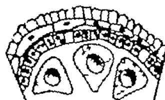
Fig 16 A young pollen grain showing a thickened exine. The nucleus is embedded in the homogeneous mass of cytoplasm X 900.

Fig 17 A bicelled pollen grain The generative nucleus is in division X 900

Fig 18. A mature, tricelled pollen grain showing a large vegetative cell and two sperms X 900

PLATE II




5 6 7

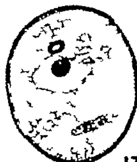



8 9 10






11 12 13 14 15

16 17 18

Explanation of Plate III

Figs 19-31 Showing various stages of megasporogenesis and development of female gametophyte.

Fig 19 An ovule in L. S. showing a single hypodermal archesporium X 900

Fig 20 An ovule in L. S. bearing multiple archesporia X 1000

Fig 21 L S. of an ovary showing the orientation of ovule at the megaspore tetrad stage. Note the 3 degenerating and a single functional megaspore X 900

Fig 22 Deep seated and fairly elongated megaspore mother cell X 900.

Fig 23 A linear tetrad of megaspores X 900

Figs. 24 & 25 A bi-nucleate embryo sac. In Fig 24 it is capped by the remains of 3 micropylar megaspores X 900

Fig 26 A 4-nucleate embryo sac X 1000

Fig 27 An 8-nucleate embryo sac (Microdissected) X 1000

Fig 28 An organized 7 celled 8-nucleate embryo sac (Reconstructed) X 900

Fig 29 A fertilized embryo sac showing a zygote, a primary endosperm nucleus and an antipodal complex X 900

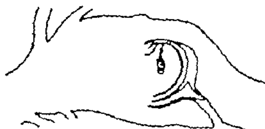
Fig 30 The chalazal region of an unfertilized embryo sac showing the greatly inflammated antipodal cells.

Fig 31 L. S of the upper half of the developing caryopsis showing persistent antipodal cells capping the endosperm X 70

PLATE III



19



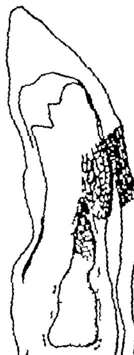
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29



2.

Explanation of Plate II

Figs 32 & 33 Showing twin embryo sacs and t in J m

Fig 32 A twin embryo sac of which one i fert li d l unfertilized Note a mass of antipodal c ll t i each embryo sac

Fig 33 A tv in seedling arising from a s ngl ed

PLATE IX



Explanation of Plate V

Figs. 34-40 Pollination and different stages in the development of endosperm

Fig. 34 A branch of the tassel showing the state of anthers at the time of dehiscence (Diagrammatic)

Fig 35 Free nuclear endosperm × 70

Fig 36 Cellular endosperm Wall formation starts from the periphery. The central region of the endosperm is still free nuclear Note also the globular embryo × 70

Fig 37 L. S of young caryopsis showing lateral and distal lobing of the endosperm × 40

Fig 38 The greatly elongated endosperm cells lying towards the placenta × 90

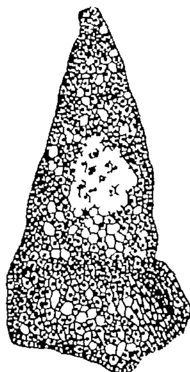
Fig 39 A part of the developing endosperm Cells of the peripheral layer showing active divisions × 900

Fig 40 A cell from fully mature endosperm, completely packed with starch grains that are simple × 900

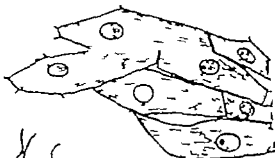
PLATE V

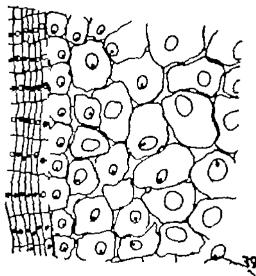


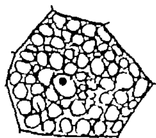












Explanation of Plate VI

Figs. 41-48 Stages in the development of the embryo.

Fig 41 First division of the zygote X 900

Fig 42 Embryo having differentiated into anterior posterior and distal lobes. Note also the long suspensor X 900

Fig 43 A developing embryo in L. S showing the coleoptile and plumule initials X 900

Fig 44 A mature embryo showing vascular supply to various parts.

Fig 45 T S of embryo passing through the region of coleoptile X 900.

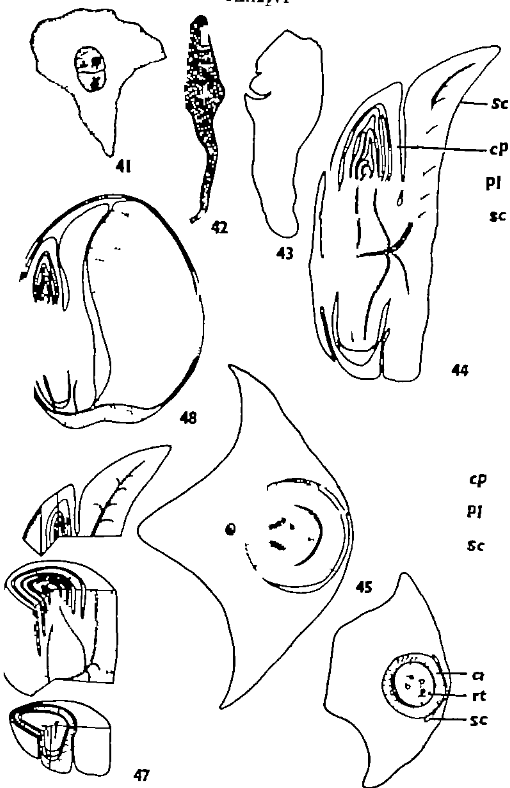
Fig 46 T S of embryo through the region of coleorhiza X 900.

Fig 47 Diagrammatic perspective representation of an embryo showing the arrangements of its parts exposed by longitudinal and transverse cuts.

Fig 48. A mature caryopsis in L. S. showing orientation of the embryo and endosperm X 70

Abbreviations cp coleoptile cr., coleorhiza pl., plumule rt., root sc., scutellum

PLATE VI



Explanation of Plate VII

Figs. 49-52. Fruit and seed of *Euchlaena mexicana*

Fig 49 *Euchlaena grain* covered by the hardened glumes and a part of the rachis (Diagrammatic)

Fig 50 An exposed naked grain of *Euchlaena* (Diagrammatic)

Fig 51 Peripheral region of the caryopsis in L. S showing the inner layer of the pericarp and the outer layer of the inner integument on the road of degeneration X 900

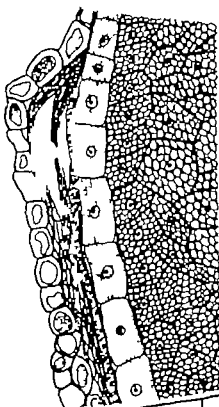
Fig 52 L. S. of the peripheral region of the caryopsis showing degeneration of inner integument and inner layer of pericarp X 900

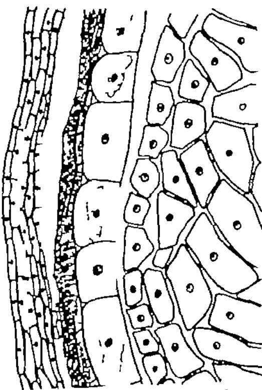
Abbreviations al., aleurone layer end., endosperm ii. inner integument ne., nucellar epidermis pe. pericarp.

PLATE VII









AN AGEOTROPIC MUTANT OF ANNUAL TEOSINTE AND ITS CYTOLOGICAL BEHAVIOUR

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Since De Vries published his famous mutation theory on Evening primrose in 1900 the concept of mutation, and the nature of changes brought about by them, has been considerably modified. These days the term mutation is applied to intragenic as well as intergenic alterations including cryptic structural changes in the chromosome complement. The effect of mutation may be either physiological or morphological bringing about some modification in habit, shape, size or colour of either a particular organ or the organism as a whole. For instance, plants in general are negatively geotropic. But quite a number of instances are on record where the mutated plants become indifferent to gravitation. Ramiah & Parthasarthy (1936) and Jones & Adair (1938) have reported such ageotropic mutants in paddy Overbeek (1936) has described some prostrate *Zea* mutants as lazy Jenkins & Gerhardt (see Overbeek, 1936) attribute this lazy character of maize to weakness of culm. Overbeek (1936) on the contrary holds that gravitational indifference rather than structural weakness accounts for such a growth habit. In a later publication he (Overbeek, 1938) reports that gravitational indifference (ageotropy) is due to disturbance in the uniform distribution of auxin which is known to cause tropic curvatures. The prostrate stems of lazy maize get straight when their under side is subjected to an application of napthelene acetic acid in lanolin. Evidently the 'lazy gene interferes with the auxin distribution in the stem. Recently Deshpande & Jeswani (1952) have described a diageotropic mutant in *Cajanus cajan*. In this case, only the main stem and secondary branches bend down while branches of the other order which bear inflorescences grow normally In 'lazy' rice mutant also at the time of heading or just before it, the culms sometimes bend up (Jones & Adair 1938)

The present paper deals with a "lazy" plant of annual teosinte. Seeds of teosinte were procured from Norman, Oklahoma, U S A., and were sown in pots in 1959 Eight inches long seedlings were transplanted in plots. One seedling started behaving differently from the rest and grew prostrate along the surface of the ground (Figs. 1 & 2) Even at the time of flowering its culms did not show any tendency to rise and inflorescences grew quite appressed to the soil. Due to this indifference towards gravitation at all stages of development this mutant has been termed as an "ageotropic mutant"

The mutant produced both male as well as female inflorescences like normal teosinte. However it was observed that no seeds developed normally

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Richardson (1936) attributes the failure of chromatid separation and lagging of chromosomes to weaker centromere charge. These abnormalities resulted in an irregular anaphasic distribution. Occurrence of laggards at anaphase and telophase II (Fig 10) was quite frequent The number of laggards at anaphase varied from 1-4 per cell. The laggards were either whole chromosomes or chromatids. Ultimately most of these laggards were incorporated in the microspores where they formed micronuclei (Fig 11) Microspores with as many as 4 micronuclei have been observed. The microspores soon degenerated and no viable pollen grains were formed. What is the part played by these micronuclei in the sterilisation of the microspores is, however not clear

As already said some of the dyad cells did not undergo second mitotic division at all The nuclei in such cells greatly elongated and ultimately fragmented into two The cytoplasm of these cells started furrowing from the periphery The furrows advanced towards the centre (Figs. 12 & 13) and finally divided the cell into two cells with nuclei of varying sizes. Two or three such direct divisions took place in quick succession to produce polysporads with 5-10 cells of unequal size (Fig 14) During this process of furrowing sometimes enucleate pieces of cytoplasm were also cut in the cells. Kumar & Abraham (1941) have described such direct divisions through furrowing of cytoplasm in sterile plants of *Sesamum orientale* Their report, however differs from the present one in that the furrowing of cytoplasm in *Sesamum* takes place after both the divisions were over The present finding of the occurrence of several consecutive direct divisions of the dyad cells in abnormal cases, instead of a single mitotic division explains one other mechanism of the origin of polyspory in plants.

Summary

A natural ageotropic mutant in annual teosinte has been described The mutant was found to be completely male sterile. Cytological studies revealed that the first division of meiosis was normal except for the occasional precocious separation of one or two bivalents at diplotene, diakinesis and metaphase In certain cells the chromosomes displayed lot of stickiness and clumping The second division was highly irregular showing laggards at anaphase and telophase and micronuclei at the microspore stage. Certain dyad cells instead of undergoing a single second mitotic division showed several, consecutive direct divisions. This resulted in polysporads each with 5-10 cells of varying sizes. All the microspores degenerated and no viable pollen grains were formed.

References

1 Beadle, J W (1932) A gene for sticky chromosomes in *Zea mays* Z. indukt. Abstamm-U-Vererblehre. 63 195-217

2. Darlington C. D & Upcott M. B. (1941) Spontaneous chromosome change. *Jour Genet.* 41 297 338.

3. Deshpande, R. B. & Jeswani, L. M. (1952) A prostrate mutant in Pigeon pea [*Cajanus cajan* (L) (Millsp)]—a possible soil conservation plant. *Ind. Jour Genet. & Pl. Breeding* 12 50-51

4 GILES, N (1941) Spontaneous chromosome aberrations in triploid *Tradescantia* hybrids Genetics 26: 632-649
5 JONES, J W & ADIAR C. R. (1938): A 'lazy' mutation in rice. *Jour Hered.* 29: 315-320
6. KUMAR, L.S.S. & ABRAHAM, A. (1941): A cytological study of sterility in *Sesamum* ... *Ind Jour Genet. & Pl. Breeding* 1 41-60.
7 LONGLEY A. E. (1941): Chromosome morphology in maize and its relatives. *Bot Rev.* 7: 263-289
8. OVERBEEK, J VAN (1936): Lazy ageotropic form of maize. *Jour Hered.* 27: 93-96.
9 OVERBEEK J VAN (1938) Lazyness' in maize due to abnormal distribution of growth hormone. *Jour Hered* 29: 339-341
10. PALIWAL, R. L. (1953): A new technique for the microdissections of embryo sacs of the Santalaceae. Phytomorph. 3: 118-121
11 RAMIAH K. & PARTHASARATHY N (1936): An ageotropic mutation in X-rayed rice. Curr. Sci 5 135-136.
12 RICHARDSON M. M (1936) Structural hybridity in *Lilium martagon album* X *L. hansonii* *J Genet.* 32: 411-450
13 TULPULE, S. H. (1959): Cytological basis for a case of sterility in an interspecific wheat hybrid. *Ind. Jour Genet. & Pl Breeding* 19: 191-198
14 WALTERS, J L. (1956): Spontaneous meiotic chromosome breakage in natural populations of *Paeonia californica* *Amer Jour Bot.* 43: 342 354
15 WALTERS, M. S (1950) Spontaneous breakage and reduction of meiotic chromosomes in hybrid *Bromus trinii* X *B maritimus* Genetics 35: 1 37

EXPLANATION OF FIGURES

Explanation of Plate No I

Figs. 1 2. Showing the habit of the ageotropic mutant of annual teosinte

PLATE No I



Explanation of Plate No II

Figs 3-8 Meiosis in the ageotropic mutant of annual teosinte Figs. 3-6 X 2100 Figs. 7 & 8 X 2400

Fig 3 Pachytene chromosomes showing knobs.

Fig 4 Diakinesis showing 10 bivalents.

Fig 5 Diakinesis, showing 8 bivalents and one quadrivalent.

Fig 6 Three bivalents showing secondary association.

Fig 7 Metaphase I showing 9 bivalents and 2 univalents, one at each pole

Fig 8. Metaphase I showing 8 bivalents and 4 univalents.

Explanation of Plate No III

PLATE No II



Figs 9-14 Second division in the ageotr pic mutant of tomato Fig 9 × 2400 Figs. 10 & 11 × 1600 Figs. 12 & 13, × [illegible] Fig 14 × 1680

Fig 9 Prophase II showing 10 chromosomes of one of the dyad cells

Fig 10 Telophase II showing 2 laggards in one of the dyad cells

Fig 11 A microspore tetrad with 2 micronuclei in one microspore and one in the other

Fig 12 A dyad cell undergoing the process of furrowing. Note [illegible] longated nucleus with two nucleoli

Fig 13 A dyad cell showing furrowing and nuclei formed by direct division. Also note a micronucleus in one cell.

Fig 14 A polysporad with 6 microspores

PLATE No III



MORPHOLOGICAL AD ANATOMICAL STUDIES IN ISOETES AND OTHER RELATED GENERA

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The Lycopsida is a unique class among the Pteridophytes. Although it has got a long and extended fossil history at present it is represented by only four living genera e.g., *Lycopodium*, *Phylloglossum* *Selaginella* and *Isoetes* beside the newly discovered genus *Stylites* These living representatives have some common characters but in certain other features they markedly differ from one another and so every one of them has been given the status of an order or a class. Among these the curious *Isoetes* holds an enviable position and has many points in its anatomy about which we do not have quite clear ideas For instance, we lack exact information about the organization of shoot apex, secondary growth of the axis, leaf initiation, structure and morphology of rhizomorph and organisation of root apex, etc.

The genus *Isoetes* has attracted the attention of botanists for a very long time. Von Mohl (1840) Hofmeister (1862) were the earliest workers to work out its anatomy Later on Scott & Hill (1900) Smith (1900) Lang (1910 1915) and West & Takeda (1915) made some valuable contributions to our knowledge of some anatomical and morphological aspects of some species of this genus. There has been little anatomical work on Indian species of *Isoetes* Whatever has been done is mostly confined to sporogenesis and cytology (Ekambaram & Venkatanathan, 1933 Abraham & Ninan, 1958) It was primarily with the object of getting some more information about certain points in the anatomy of common Indian species *I. coromandelina* that the present study was undertaken. The results on the shoot apex of *I coromandelina* (Bhambie 1957) were encouraging and so a study of shoot apices in *Lycopodium* and *Selaginella* has also been undertaken

The present investigation includes 32 species of class Lycopsida of which 10 belong to *Isoetes*, 12 to *Lycopodium* and 10 to *Selaginella* The anatomy of the locally occurring species *I coromandelina* has been worked out in detail but accounts of other species of *Isoetes* are not so detailed for want of adequate amount of material. In *Lycopodium* and *Selaginella* only the organization of shoot apex has been studied.

In *Isoetes coromandelina* the shoot apex is situated in a deep conical depression of the axis and consists of a group of apical cells distinguishable into two more or less demarcated regions—an outer layer having chiefly anticlinal divisions and an inner dome of irregularly dividing cells. Below this apical protuberance

This is an abstract of the thesis submitted and approved for the Ph. D. degree of the Agra University in the year 1960.

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there is an undifferentiated procambial column. The differentiation of tissue seems to be centrifugal though some irregularity has sometimes been observed due to stunted growth of the plant.

The vascular structure of the axis is similar in all the species of *Isoetes* and the stele is differentiated into two parts, an upper stem stele which sends off leaf-traces only and a bi- tri- or tetra-radiate rhizomorphic stele which has got its own meristem and gives off only root traces. Morphologically the rhizomorph appears to be stem-like in nature as its anatomy is similar to the stem part of the axis and has its own meristem upon which roots are borne in a regular order

As regards the structure of stele, the centre is occupied by xylem elements and surrounded by a few layers of parenchyma. Next there are some layers of primary phloem which are irregularly arranged and have pit-like processes on all their walls. The cambium is peculiar in so far it originates outside the primary phloem, intrafascicular cambium being altogether absent in the species studied here It cuts off secondary parenchyma on its outerside and some mixed tissue on its innerside, the nature of which is highly controversial. This latter tissue is found to consist mainly of specialized sieve cells with sieve areas on all the walls and parenchyma with occasional occurrence of some lignified cells. The sieve cells forming secondary phloem have white glistening walls, clear appearance of their contents, deposition of callose around the pores and absence of nuclei and ordinary starch grains which occur commonly in other cells of *Isoetes* The presence of callus in phloem elements and lignin in lignified cells has been recognised by their microchemical tests with Rosolic acid, Aniline blue and Alcoholic phloroglucin respectively

The leaf in *I coromandelina* originates from a group of a few superficial cells. To begin with every cell of this primordium is meristematic but after the formation of ligule velum and sporangium the basal cells mature first. The supraligular region remains meristematic much longer and afterwards the maturation of cells starts from base upwards until the whole leaf is mature. The ligule develops from a single adaxial cell of the young primordium which divides twice by transverse divisions and forms a three-celled structure The upper two cells then by further vertical divisions form a plate of eight cells in which further growth becomes irregular while the remaining basal one by irregular divisions gives rise to the glossopodium. In mature condition the ligule has two parts, a lamina and a bilobed glossopodium embedded in the leaf tissue A few tracheid like cells which are in continuation with the vascular bundle of leaf surround the glossopodium on all sides. Another characteristic structure of *Isoetes* leaf is the velum. It develops from a group of cells between the ligule and leaf base before any sign of sporangium is seen and thus has an independent origin In *I coromandelina* the velum has only an upper labium which grows towards the ligule side but in other species it may have two labia—an upper and

a lower The lower labium covers the sporangium partly (*I sampathkumarani*) or fully (*I sahyadrii*) and is of variable size in different species. The sporangium develops by a group of cells from the tissue which is situated between the velum and the leaf base. Its development is typically eusporangiate. In mature condition the sporangium contains large number of spores and is traversed by several trabeculae at places, which are not partition walls but pillars or columns of sterile cells projecting from one wall towards the other The sporophylls are exclusively megasporangiate and the megaspores show several abnormalities though some shrivelled sporophylls containing smaller spores are sometimes encountered. These spores are, however quite different from the microspores observed in some other species like *I lacustris I japonica, I asiatica* and *I sp* Presence of a few young plants with 7 or more leaves and the absence of microspores point out that the megaspores develop apogamously The absence of microspores in this species has also been recorded previously

The formation of microgametophyte in *I lacustris* confirms the work of Liebig (1931)

The anatomy of leaf is simple, it is traversed by a single vascular bundle but at the back of the sporangium in a sporophyll its tracheids spread out and form a network, which again fuse and form a compact structure above the sporangial region.

The roots develop acropetally in a perfect order On one cortical lobe of the axis, the roots coming out from one half of the two rhizomorphic lobes are arranged as the stelar lobes of rhizomorph coincide with the furrows of the axis. The seasonal roots arise endogenously and develop from a group of cells just below the rhizomorphic stele. In mature condition the root promeristem can be distinguished into two regions—a central cylinder or core and an inverted cup-shaped meristem or 'mantle which is present upon the central cylinder The meristematic cells of the core give rise to the stele, while the mantle forms the whole cortex, epidermis, columella and the side tissues of root cap The branching of root is dichotomous. The anatomy of root is peculiar though simple. It is traversed by a monarch eccentrically placed stele. The inner cortex round the stele of root disorganises very ea ly in the ontogeny and forms a C' shaped cavity a chara teristic feature of some of the fossil lycopods.

The systematic position of *Isoetes* has been discussed under two heads i.e., (i) affin ties with Lycopsida and (ii) affinities with Pteropsida, and on the basis of a comparison of rhizomorph of *Isoetes* with *Stigmaria* its adaxially developing eusporangiate sporangium, presence of ligule and parichnh like structures and the characteristic structure and arrangement of roots, its place in the class Lycopsida is further confirmed.

In *Lycopodium*, only the organization of shoot-apex has been studied and it has been brought out that the stem apex consists of a hump of meristematic cells distinguishabl into an outer layer covering an inner dome. A few centra

cells of the outer layer in certain species of *Lycopodium* are somewhat bigger and less active. In form the apex varies in different species from a flat-topped structure to a strictly conical one

In *Selaginella* two types of shoot apices are met with (i) dorsiventrally flattened apices (ii) radially symmetrical apices. There is a group of conspicuous apical cells at the top which are in the form of a plate in the dorsiventrally flattened apices and so a single cell is seen when this plate is cut vertically In radially symmetrical apices, on the other hand, a central group of cells is visible in both the vertical longitudinal and horizontal longitudinal sections. These apical cells are generally less cytoplasmic and divide rarely in comparison to the cells of the lateral flanks which form an outer layer This layer of lateral flanks divides both anticlinally and periclinally The cells of the tissue present inner to the flanks also divide actively in different planes.

This analysis of the data on the organization of shoot apex in all the three genera viz,. *Isoetes* *Lycopodium* and *Selaginella* shows that all of them are highly specialized in their own way The rare occurrence of periclinal divisions in the superficial layer of *I coromandelina* indicates that *Isoetes* is probably more highly evolved than the other two genera in this respect.

REFERENCES

1 Abraham, A. & Ninan, C. A. (1958) Cytology of Isoetes Curr Sci. 27 60-61.

2. Bhambie S. (1957) Studies in Pteridophytes I The Shoot apex of *I Coromandelina* L. Jour. Indian Bot. Soc. 36 491-502.

3 Dhamberam, T & Venkatanathan T N (1933) Studies on *Isoetes Coromandelina* L. I Sporogenesis. Jour Indian Bot. Soc. 12 : 191-225

4 Hofmeister W (1862) The Germination Development and Fructification of Higher Cryptogamia. London.

5 Lang W H. (1910) On the Stock of Isoetes. Report of British Advancement of Science Sheffield. 781

6. Lang, W H. (1915) Studies in the morphology of *Isoetes* I The general morphology of the stock of *Isoetes Lacustris* Mem Proc. Manchester Lit. Phil Soc. 59 1-28

7 Liebig, J (1931) *Ergaenzungen und Entwicklung* Ungeschlechtlicher von *Isoetes lacustris* L. Flora 125 321 358.

8. Mohl, H. Von. (1840) *Uber den Bau des Stammes von Isoetes lacustris* *Linnaea* (Verm. Schriften Bot. Inh., Tubingen, 1845.

9 Scott, D.H. & Hill, T G (1900) The structure of *Isoetes hystrix* Ann. Bot. 14 413-454

10. Smith R. W (1900) The structure and development of the sporophylls and sporangia of *Isoetes* Bot. Gaz. 29 225-258 323-346.

11. West, C. & Takeda, H (1915) On *Isoetes japonica* A. Br Trans. Linn. Soc Lond. Bot. 8 333-376

SYNERESIS OF SODIUM OLEATE GELS IN A FRACTION OF TURPENTINE

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It is known that number of gels of organic substances, such as those of germ nin and viscose exhibit the phenomenon of syneresis. Prasad and co-workers (1) found that the gels of sodium oleate in pinene and xylene also synerise to an appreciable extent. They have measured the rate of syneresis of these gels containing different amounts of sodium oleate at several temperatures, and have studied the effects of surface area of gels and of addition agents on the rate of syneresis.

The authors found that sodium oleate forms gels in a fraction of turpentine collected at 164° C, and these gels synerise like sodium oleate gels studied by Prasad and co-workers. The present investigation deals with the study of the effect of temperature on the rate of syneresis of these gels containing different amounts of sodium oleate.

EXPERIMENTAL

(a) *Distillation of Turpentine*

The turpentine, purchased from local market, was distilled at 32 cm. atmospheric pressure, and the fraction separating out at this pressure was collected. The physical characteristics of this fraction were measured and are given below —

Density	=0.865 at 16° C.
Refractive Index	=1 4715 at 16° C
Surface tension	=24 64 Dynes/cm. at 16° C.
Viscosity	=16.9 Dynes/square cm at 16° C.
Boiling point	=164 C.

(b) The sodium oleate used was of the B. D H. quality

(c) *Preparation of gels*

Gels of sodium oleate in the fraction of turpentine, called hereafter as liquid A, were prepared in pyrex glass test tubes of th same internal diameter Known amounts of sodium oleate were weighed out in several test tubes and 10 c. c. of the liquid A were added to each of them. The test-tubes were then placed in an oil bath maintained at 168° C and the contents were stirred from time to time till all the soap was dissolved. The test tubes were wiped to remove the adhering oil of the bath and then placed in a water thermostat maintained at

a constant temperature. The solutions in each test tube set to gel after some time the setting was determined from the fact that the contents of the test-tubes did not show any tendency to flow out when they were inverted. The time when the gel had just been formed was taken as the starting time for the measurement of the rate of syneresis.

(*d*) *Measurement of the rate of syneresis*

This was measured by the method of Prasad and co-workers as follows:—

After a certain interval of time one of the test tubes was removed from the water thermostat and the amount of liquid exuded was removed by slowly tilting the test tube and allowing the syneretic cum to drop out into another container the last traces of syneretic cum were removed by carefully introducing small rolls of filter paper vertically into the test tube. The rolls of filter paper were changed when they were found to be saturated with the syneretic liquid The introduction of filter papers was stopped when it was considered that no more exuded liquid remained in the test-tube. The test-tube with its container which was weighed before, was weighed again, and the loss of weight due to the removal of exuded liquid in the given time was determined. Another test tube was removed after another interval of time, and the amount of syneresis was determined in the same manner as with the gel in first tube. This process was repeated with the gels formed in several test-tubes at known different intervals of time. The results obtained are given in Tables I II and III in which the following notations have been used —

T —Temperature of observation in degrees centigrade

t —Time in seconds after the setting of gels.

x —The amount of syneresis in grams.

Km —The unimolecular constant $= \frac{2.3}{t} \log \frac{a}{a-x}$

Q —Weight in grams of sodium oleate in the gel.

a —The weight in grams of the solvent in the gel.

TABLE I

T=20° C

a=8.65

t	Q=0 0900			Q=0.1136			Q=[illegible]		
	x	$\frac{x}{t} \times 10^4$	$\frac{Km}{10^4}$	x	$\frac{x}{t}$ 10⁴	$\frac{Km}{10^4}$	x	$\frac{x}{t}$ 10⁴	Km × 10⁴
3600	0 3284	9 12	10 733	0.2716	7 54	8.800	0.1908	3.50	6 1[illegible]
5400	0 3918	7 25	8 561	0.3444	6.37	7 496	0.2202	4.07	4 7[illegible]
7200	0 4608	6 40	7 570	0.3678	5 11	6.037	0.2322	3 2[illegible]	3 1[illegible]
9000	0.5208	5 78	6 874	0.3696	4 11	4 855	0.2448	[illegible]72	[illegible]
10800	0 5774	5 35	6.388	0 3699	3 42	4.016	0.2502	[illegible]31	[illegible]
12600	0.5784	4.58	5 476	0.3702	2.93	3 468	0.[illegible]350	2.01	[illegible]
14400	0.5788	4 02	4 791	0.3710	2.57	3 034	0.2560	1 77	[illegible]

TABLE II

T=30°C.

n=8.65

t	Q=0.0900			Q=0.1156			Q=0.1800		
	x	$\frac{x}{t}\times 10^4$	Km × 10^4	x	$\frac{x}{t}\times 10^4$	Km × 10^4	x	$\frac{x}{t}\times 10^4$	Km × 10^4
3600	0.3076	8.54	10.030	0.2202	6.11	7.155	0.1038	2.88	3.322
5400	0.3596	6.66	7.837	0.3222	5.96	7.027	0.1388	2.57	2.981
7200	0.3872	5.38	6.336	0.3296	4.57	5.398	0.1548	2.15	2.491
9000	0.4352	4.83	5.750	0.3408	3.78	4.472	0.1674	1.86	2.172
10800	0.4718	4.37	5.196	0.3480	3.22	3.790	0.1740	1.61	1.874
12600	0.4818	3.82	4.545	0.3502	2.77	3.267	0.1782	1.41	1.642
14400	0.4868	3.38	4.009	0.3518	2.44	2.875	0.1820	1.26	1.469

TABLE III

T=60° C

n=8.65

t	Q=0.0900			Q=0.1156			Q=0.1800		
	x	$\frac{x}{t}\times 10^4$	Km × 10^4	x	$\frac{x}{t}\times 10^4$	Km × 10^4	x	$\frac{x}{t}\times 10^4$	Km × 10^4
3600	0.3010	8.36	9.775	0.2116	5.87	6.900	0.1004	2.78	3.194
5400	0.3480	6.44	7.581	0.2978	5.51	6.474	0.1298	2.40	2.811
7200	0.3654	5.07	6.005	0.3158	4.38	5.175	0.1502	2.08	2.427
9000	0.3746	4.16	4.906	0.3368	3.74	4.395	0.1624	1.80	2.095
10800	0.3804	3.52	4.152	0.3398	3.14	3.705	0.1688	1.56	1.810
12600	0.3846	3.05	3.596	0.3408	2.70	3.176	0.1704	1.35	1.569
14400	0.3852	2.67	3.162	0.3416	2.37	2.795	0.1728	1.20	1.389

DISCUSSION OF RESULTS

(a) *Effect of time*

It will be seen from Tables I, II and III that the amount of syneresis increases with time fairly rapidly in the early stages and subsequently the rate of syneresis slows down considerably. These conclusions are brought out very clearly from some of the curves shown in figure 1. The curves (not shown) obtained by plotting the values of $\frac{x}{t}$ against t, which are in all cases hyperbolic in appearance, lead to the same conclusions.

It will be seen that all curves in Fig 1 are smooth rising ones and are definitely not S-shaped this shows that the syneresis of the gels studied in this investigation is not an autocatalytic process as found by Ferguson and Applebey (2) in the case of silicic acid gels.

On keeping the gels for a very long time (several months) it was observed that almost all the liquid is exuded and only a small flake of solvated soap settles down at the bottom. However no regular estimation of the rate of syneresis during this period was made in this investigation.

In order to examine the truth of the findings of Lipatov and Korobova (3) made in the case of geranium gels, the values of the unimolecular constant (Km) were calculated for different intervals of times. It will be seen from the Tables I II and III that they are not constant but decrease as the time interval is increased thereby showing that the law of unimolecular reaction is not obeyed in this case. However on plotting the values of Km against those of $\frac{1}{t}$ it is found that all the plotted points lie on a straight line hence it appears that there exists a linear relation between $v = \frac{x}{t}$ and Km. Writing this relation in the form

$$v = \frac{x}{t} = \left(a+\frac{1}{b}\right)\frac{1}{t}\log\frac{a}{a-x} - \frac{k}{b}$$

where k and b are constants, it will be found that the rate of syneresis is given by $\frac{dx}{dt} = \frac{k(a-x)}{1+bx}$ Further consideration of this empirical relation is necessary as it may lead to the understanding of the mechanism of syneresis in a quantitative manner

Bell and Cameron (4) have shown that the relation $X^n = kt$, where X is the height through which the liquid rises in a strip of filter paper in time t and k and n are constants the value of n being equal to 2, is applicable to the rise of liquids through gels hence it may also be applicable to the reverse process, that is the exudation of liquid by gels. The application of this relation was examined by plotting the values of log x against log t. It has been found that the plotted points lie on straight lines This would show that the syneresis of the gels studied by authors, obeys this relation also though not exactly since the value of n is not equal to 2

(b) *Effect of temperature*

It will be seen from Tables I II and III that the syneresis decreases with an increase in temperature This conclusion can also be seen from the $\frac{x}{t}$ curves for different temperatures. The values of Km at a given interval of time are greater for lower temperatures and decrease as the temperature increases

The values of $\frac{x}{t}$ at different temperatures for the same concentration of the soap in the gels have been plotted against the corresponding values of Km the plotted points have been found to be more or less on the same straight line. In order to find whether the relation X =KT holds good at all temperatures studied, the values of log x were plotted against those of log t, and in all cases the plotted points were found to be on straight lines.

(c) *Effect of concentration*

Tables I, II and III also show that the amount of syneresis in any given interval of time is greater for the dilute gels than for the concentrated ones. This conclusion can also be seen from the $\frac{x}{t}$—t curves for gels of different concentrations.

Gapon (5) has found that the amount of syneresis from gels of different concentrations at the same interval of time is given by relation $X_s = H(P_0\ P)$ where X_s is the amount of synereticum in percentage of the initial quantity of the solvent for the concentration of the solute P P_0 is the concentration at which no syneresis takes place, and H is the syneresis constant. It follows from this relation that the plot of X against Q quantities which are analogous to X_s and P should be straight lines. The curves obtained are far from being straight lines, showing thereby that Gapon's equation is not applicable to the syneresis of sodium oleate gels studied in this investigation.

Summary and Conclusions

The syneresis of sodium oleate gels formed in a fraction of the liquid obtained by the distillation of turpentine has been studied by th method of Prasad and co-workers, and the effects of time, temperature and concentration of the gel-forming system on the syneresis have been investigated.

The rate of syneresis has been found to decrease with an increase in time The graphs of amount of syneresis against time are smooth rising curves The process of syneresis of the gels studied in this investigation does not follow the unimolecular law of chemical reactions the values of the unimolecular constant decrease with increase in time. Syneresis observed in this case can also not be said to be the exact reverse of imbibition of liquids by gels, but seems to be similar to it, since the value of n is not exactly equal to 2 however the plots of log x against log t are very nearly straight lines.

Increase of temperature decreases syneresis. Increase in concentration also causes a decrease in syneresis.

All the results obtained on the syneresis of the gels studied in this investigation are in conformity with those obtained by Prasad and co-workers and can

be very satisfactorily explained on the basis of the theory of gel formation developed by these wrokers

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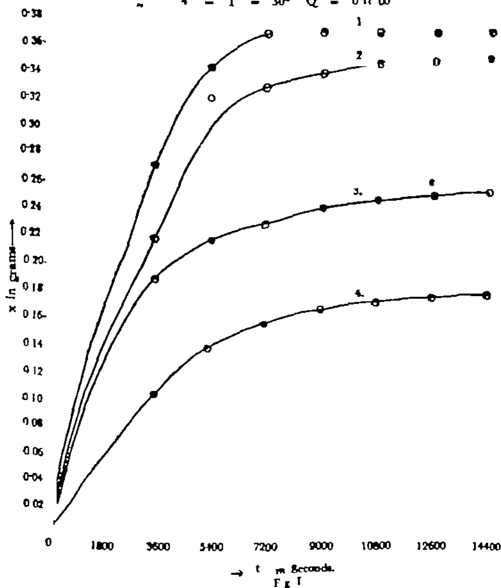
References

1 Prasad and Co-workers Prasad and Sundaram, Indian Acad. Sci, Vol. XXXIII, 1951.
Prasad, Hattiangdi and Mathur Proc. Indian Acad. Sci., 1945, 21A, 90 105

2. Fergusson and Applebey Trans. Farad. Soc., 1930, 26, 642.

3. Lipatov and Korobova : Zeit. Anorg Allgem. Chem., 1930, 194, 377

4 Bell Cameron : J Phys. Chem., 1906, 10 658; Kolloid Z., 1908, 2, 20.
Gapon Ukrainskii Khim Zhur., 1930 Sci. Pt. 5 59.



Curve 1 — T — 20° Q — 0·1136
" 2. — T — 30° Q — 0·1136
" 3 — T — 20° Q — 0·18 00
" 4 — T — 30° Q — 0·18 00
1
2
3.
4.
0·38
0·36
0·34
0·32
0·30
0·28
0·26
0·24
0·22
0·20
0·18
0·16
0·14
0·12
0·10
0·08
0·06
0·04
0·02
0
1800
3600
5400
7200
9000
10800
12600
14400
x in grams →
→ t in Seconds.

Fig. 1

SOME ASPECTS OF ELECTROCHEMICAL INVESTIGATIONS IN FUSED POTASSIUM CHLORIDE—LITHIUM CHLORIDE AS SOLVENT

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SOLVENT AND FUSED SALT METHODOLOGY

This thesis describes some aspects of the electrochemical investigations in fused eutectic mixture of potassium chloride (41.2 mole per cent) and lithium chloride (58.8 mole per cent) used as a solvent. The working temperature was 450° C which was controlled within ±2° by an electronic temperature controller. The choice of the solvent was based upon its thermal stability absence of strong acidic or basic properties, and, the availability of a wide potential span of over 2 volts, limited by the anodic oxidation of Cl^- and the cathodic reduction of Li^+. However this potential span was available only if the solvent was prepared under controlled conditions. The difficulties encountered in the preparation of the solvent have been enumerated and the procedure employed has been described. The purity of the solvent was ascertained by taking a polarographic residual current for an acceptable melt using a platinum microelectrode (P. M. E.) with an area of 0.0013 cm² there was no wave at about —1.0 volt and the residual current up to a potential of —2·0 volt *vs* platinum reference was 2.3 microamperes.

The reference electrode employed was a platinum foil in contact with Pt(II) solution of known concentration in the solvent. In all the experiments Pt(II) was 0.1 *M*., the required amount having been generated in the solvent before starting an experiment by anodic dissolution of a thick platinum foil.

IMPEDANCE MEASUREMENTS AT A SOLID MICROELECTRODE

Impedance measurements enable one to have an insight into the processes occuring at the metal electrode/electrolyte solution interface.[1] The technique has found most applications in the study of the kinetics of electrode processes in aqeous medium using dropping mercury electrode (D. M. E.) as a polarizable cathode. The extension of these studies to fused eutectic KCl-LiCl as solvent at 450° C using platinum microelectrodes was done to obtain, hitherto unavailable, quantitatively significant information about the kinetics of discharge of metal ions in this solvent.

For impedance measurements the Wien's bridge was employed. By a potentiometric arrangement and employing a platinum foil as a working elec

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trode, the microelectrode was polarized (given a negative bias potential its potential with respect to the platinum reference being measured with a potentiometer The over all impedance of the system was measured at frequencies ranging from 100 to 3000 cycles/sec of the a. c. applied to the bridge, and at various microelectrode potentials.

The gross impedance of the system comprising of the faradaic or the reaction impedance (which is best regarded as a series combination of a resistance R_r called polarization resistance, and a capacity C_r, termed pseudo-capacity), the double layer capacity at the Pt/melt interface (C_{dl}) and the solution resistance (R_e) was measured as a series combination of a resistance, R_x, and a capacitance, C

From this the reaction impedance was isolated by the method of Laitinen and Randles.[1]

RESULTS OF IMPEDANCE MEASUREMENTS

(A) *Residual Impedance*—Impedance measurements were first carried out in the solvent in potential range —0.3 to —1 8 volt *vs* platinum reference which is far removed from the decomposition potential of the solvent or the anodic dissolution potential of platinum. Since no faradaic impedance is involved, C_x should correspond to C_{dl} and R_x to R_e, and the values of both should be independent of the frequency of the a.c. However both were found to be strongly frequency dependent.[2] Similar frequency dispersion in residual impedance as this will be referred to, in aqueous and non aqueous media, had been observed by other workers also who used solid microelectrodes, as presently employed So far it has not been possible to explain this the causes attributed by different workers as roughness of the electrode surface[4,5] or its contamination in the solution[6] the formation of an oxide layer on the electrode surface which behaves as a condenser with a low leakage resistance[7] or the dielectric loss associated with a firmly attached layer of solvent[8] were closely examined and found to be inadequate in the present case.

Over the potential range of —0.5 to —1 0 volt *vs* platinum reference the plots of residual C_x at various frequencies were extrapolated to $1/\sqrt{w}=0$ or infinite frequency This corresponded to a value of 58-75μ F/cm² which is of the order observed with a D M. E. in aqueous solutions of electrolytes, and was regarded as closely approximating the double layer capacity at the Pt/melt interface. It was thus concluded that the 'residual' C_x was made up of a small *frequency independent* double layer capacity in parallel with a large *frequency dependent* component which is not dependent upon the true area of the electrode. This model, explained the observations with electrodes having 'rough' and 'smooth' surfaces.

For the purpose of isolating reaction impedance from the observed gross impedance, the value of C_{dl} was however needed It was considered [illegible]

to use the above extrapolated value. In order to take frequency dispersion into consideration the residual C_{xs} as observed at a given frequency was used instead of C_{s1}. Residual R_{xs} values were in general much higher than the ohmic resistance directly measured. At a given potential, R was measured in presence of the depolarizing ion.

(B) *Faradaic Impedance*—These measurements were carried out in presence of a depolarizing ion in the solvent the reduction of Pb^{++} Cd^{++} Ni^{++} Co^{++} and Zn^{++} was studied. The measurements were recorded under two conditions

(a) *Forward polarization*—When the microelectrode potential was gradually made more cathodic from about—0 3 volt *vs* platinum reference to few tenths volt cathodic to the equilibrium potential

(b) *Reverse polarization* —The direction of the change of potential of the (now metal coated) microelectrode was reversed, *i.e.* it was gradually made less cathodic till it was few tenths volt anodic to the equilibrium potential.

From the results of these measurements, the following general features were noted

(i) On forward polarization before the equilibrium potential was reached, the platinum microelectrode was in contact with the metal ions in the solvent and acted as a noble electrode thus did not represent a state of electrochemical equilibrium at the P M E. The impedance measurments in this potential range should be similar in all solutions since no characteristic faradaic process is involved this was found to be so the observed values being of the same order as for the solvent.

In all the cases the R plots tended towards a maximum which may be compared with a similar broad maximum in the potential range —1 1 to —1.8 volt in the residual R_x plots

(ii) In the reduction of all the above ions there was a tendency for predeposition *i.e.* the discharge of ions occuring at potentials anodic to the equilibrium potential the observed *underVoltage* varied from 0 035 volt for the discharge of Pb^{++} to about 0 158 volt for the discharge of Cd^{++}

(iii) At the equilibrium potential when the metal deposited on the electrode transformed this into a metal -metal ion electrode, a pseudo-capacity appeared in all the cases, as could be anticipated from theoretical consideration. A minimum in R and the total impedance was also shown at this potential. The equilibrium potential corresponds, by definition, to a condition that no net current is drawn through the system.

(iv) On further increasing the bias potential so that it was cathodic to the equilibrium potential a net cathodic current flowed through the system in all the cases C_{xs} rapidly decreased and R rapidly increased due to normal concentration polarization.

(v) On reversing the polarization, up to the equilibrium potential C_{x} rapidly increased and R_{s} gradually decreased though the values now at different P. M. E. potentials were slightly different than those on forward polarization due to difference in the conditions of the electrode surface in the two cases.

On further decreasing the potential so that the P. M. E. potential was anodic to the equilibrium potential, the dissolution of the metal occurred and a net anodic current flowed through the system. For this region it is seen that in all the cases, C_{xs} continues to increase rapidly while R tends to a limiting value which is of the order of 15-40 ohms in all the cases.

The variations in C_{x} and R_{s} (and since the maximal change will arise primarily from the reaction impedance hence its variation) with potential under above conditions (iii) to (v) were explained from the available theoretical considerations on faradaic impedance.[9]

Calculation of kinetic parameters

For the present case of the deposition of a metal ion on a solid microelectrode, the components of reaction impedance are given by

$$C_r = \frac{RT}{n^2F^2AC_0^{\ 1-\alpha}} \left[\left(\frac{2}{wD}\right)^{\frac{1}{2}} + \frac{1}{k}\right] \quad (1)$$

$$C_r = \frac{n^2F^2AC_0^{\ 1-\alpha}}{RT} \left(\frac{D}{2w}\right)^{\frac{1}{2}} \quad (2)$$

$$\text{or } \triangle = R - \frac{1}{wC_r} = \frac{RT}{n^2F^2AC_{ox}^{\ 1-\alpha}k} \quad (3)$$

It is also possible to express $\triangle$ in terms of exchange current, i_0

$$i_0 = nFAkC_0^{\ 1-\alpha} \quad (4)$$

$$\text{So that } \triangle = \frac{RT}{nFi_0} \quad (5)$$

From (1) and (2) the plot of R_r and $\frac{1}{wC_r}$ vs $1/\sqrt{w}$ should give two parallel straight lines the resistance plot lying over the capacitative reactance plot, the latter passing through the origin and the phase angle of the combination given by $\tan\phi = \frac{1}{wC_rR}$ should be *less* than $\frac{\pi}{4}$, this value being reached for an immeasurably fast reaction.

In the present study ϕ was always *greater* than $\frac{\pi}{4}$ so that the positions of the impedance plot were reversed, and furthermore the reactance plot missed the origin. From an earlier experience of Laitinen and Randles[3] to be faced with a similar difficulty, this anamolous behaviour was ascribed to the

adsorption of reactant ions at the electrode, the admittance to this being regarded as additional to the reaction admittance A correction was applied so that the reactance plot passed through the origin and the resistance plot lay over it and was parallel to it. The proper value of the correction was rather critical and found by several trials.

In the reduction of Pb^{++} Ni^{++} and Co^{++} the uncorrected plot were close to each other and only capacitative corrections of 1 1 0 3 and 1.3 μ F respectively were needed for the reactance plot to pass through the origin this correction also shifted the resistance plot and the two overlapped as would be the case for an immeasurably fast electrode reaction For the reduction of Zn^{++} and Cd^{++} the correction needed was a series combination of a resistance and a capacitance and the two plots were separated from each other th value of $\triangle$ being 12.5 and 3 0 ohm respectively Since the value of α is not known, k could not be calculated Under these conditions t appeared best, as Gerischer chooses to do to report an exchange current density at the concentrations employed. For Zn^{++}/Zn and Cd^{++}/Cd systems at concentrations f 7 16 $\times 10^{-4}$ moles/cm^3 the exchange current density was f und to be 18.4 and 7 7 amp/cm^2 These values are in good agreement with the values obtained by application of independent methods viz th voltage step[10] and the double pulse methods[11] by other workers whose results have been included for purpose of comparison.

The significance of the correction has been discussed.

Polarization Behaviour of Microelectrode in Fused KCl LiCl Eutectic as Solvent

These experiments were carried out to st dy th current potential behavi our of a system when the microelectrode potential was shifted slightly in cathodic and anodic directions from the equilibrium potential. For a given over voltage, $\eta(=E_{appl} \; E_{eqm})$ the current i, in terms of exchange current, i_0 is given by $=i_0 \exp \left\{\frac{\alpha nF\eta}{RT} \; -\exp(1-\alpha) \; \frac{nF\eta}{RT}\right\}$

This equation contains two observable variables i and η and two unknown variables i_0 and n If the contribution of the current by the reverse process can be considered negligible (for $\eta > \frac{0.288}{n}$ volt) the slope of log i vs η plot gives n α and the intercept on log i axis will give i However with the microelectrodes as employed considerable overvoltages could not be given and hence direct determination of n and i_0 was not poss ble In an indirect method for a given current potential curve the values of i_0 and nα were so chosen that the values of i calculated from the above for different values of η, corresponded as nearly as possible to the observed values of i. The polarization curves fo the reduction of Pb^{++} Ni^{++} and Ag showed no inflection in the region of equilibrium potential

showing that the reduction process were unmeasurably fast. For reduction of Cd^{++} Zn^{++} and Bi^{+++} the values of $n\alpha$ were estimated to be 0.30, 0.53 and 0.30 respectively the values of r were in general much higher than those obtained by the a.c. impedance technique. The anamolous polarization curve for Co^{++} has been discussed.

Chronopotentiometry in Fused KCl-LiCl Eutectic As Solvent

The fundamentals of chronopotentiometry or constant current electrolysis have been reviewed and the applications in aqueous and non-aqueous media have been surveyed. In general the quantitative interpretation is possible under the following conditions electrolysis current remains constant for the duration of electrolysis, a large excess of the supporting electrolyte is present, the concentration of the depolarizer before electrolysis is uniform throughout the solution, diffusion is the sole mode of transport of the ions, and the condition of semi-infinite linear diffusion prevails In practice the above conditions were satisfied

The basis of the method is to apply a constant current between a platinum working electrode and a microelectrode. The potential variation (with respect to a reference electrode) of the latter with time is studied.

Platinum microelectrodes of different areas and geometry were prepared by sealing appropriate B & S guage wire into Corning 0120 glass the projected areas being determined accurately by optical micrometry Electrodes 1, 2 and 3 consisted respectively of 26- 23- and 18-guage wire sealed into 6 mm. tube, flush ground and polished, the respective areas being 0.13, 0.333 and 0.81 mm^2 Electrodes 4 and 5 were cylindrical made from 26- and 18-guage platinum wire projecting to a distance of 0.648 and 1.107 mm. with total areas of 0.976 and 4.608 mm^2 respectively Electrode 6 was made from 2 mm. thick platinum foil 2.024x1.419 mm suspended by a 20 mil platinum wire

The oscillographic method of recording the potential-time curves was employed. By use of electromagnetic relays, the constant current was applied between the platinum working electrode and one of the above microelectrodes simultaneously it triggered the driven sweep of the C. R. O and the potential variation of the microelectrode was applied thorough a. d. c. amplifier to the deflection plates of the oscillograph Time-base was provided by feeding a square wave signal of known frequency to the Z-modulation input of the C. R. O The traces were photographed on 35 mm. film and measurements made from 4 5 enlargements. The arrangement enabled determination of transition time T with an accuracy of ± 2 per cent, but since in chronopotentiometry $T^{\frac{1}{2}}$ used the latter was known with an accuracy of about 1 4 per cent.

A potential-time curve for the solvent in the potential range —1.0 to —1.5

volt vs. platinum reference gave the residual $I T^{\frac{1}{2}}$ between 0.35 to 0.6x 10^{-3} amp cm^{-2} $sec^{\frac{1}{2}}$

Detailed chronopotentiometric data for different concentrations of cadmium chloride in the solvent at different current densities and with electrodes of different areas and geometry have been presented. For different concentrations the values of $IT^{\frac{1}{2}}$ were found. In each case a constant $\frac{IT^{\frac{1}{2}}}{C^0}$ which has been termed transition time constant, was calculated. Since the data with several electrodes were available the relative response of the different microelectrodes was compared. Electrodes 6 which approximates ideally to linear diffusion condition was made the basis fo this comparison. For electrodes 3 4 and 5 the condition of semi infite linear diffusion was found to hold good for electrolysis duration of about one second insp te of the differences in the areas and geometries of the electrodes deviation for electrodes 1 and 2 were shown to be due to constrained linear diffusion By plotting the different variables it was concluded that principles of chronopotentiometry were obeyed if the duration of electrolysis was about 1 2 seconds and the dimensions of the electrode were much larger than the prevelent diffusion field.

Chronopotentiometric data fo the reduction of Pb(II) Co(II) Ni(II) Tl(I) Ag(I) in fused KCl LiCl as solvent are also presented. From the earlier experience only electrode 3 was used

The average value of transition-time constants for the reduction of Cd(II) Pb(II) Co(II) Ni(II) Tl(I) and Ag(I) were found to be 0 82₄ 0 95₄, 0.90₈ 1.27₆, 0.56₄ and 0 688 × 10^{3} amp cm $sec^{\frac{1}{2}}$ per mole respectively The $IT^{\frac{1}{2}}$ vs C^0(depolarizer concentration) curves for these ions had slopes of 0 783 0.80 0 845 1 0 0.528, and 0 686 × 10^{3} amp cm $sec^{\frac{1}{2}}$ per mole from which the diffusion coefficients of these ions in this solvent were calculated to be 2 08, 2.18, 2.42, 3.43, 3 88 and 6.54 × 10^{-5} cm^2/sec

REFERENCES

1 J E. B. Randles. *Discussion Faraday Soc.* 1, 11 (1947); B. Ershler *Ibid*, 1 269 (1947) *J Phys. Chem. (U S S. R)* 22, 684 (1948) H. Gerischer *Z physik. chem.*, 198 286 (1951)

2 H. A. Laitinen and J E. B. Randles, *Trans. Faraday Soc* 51, 54 (1955)

3 H. A. Laitinen and H. C. Gaur *Technical Report to U S. Office of Ordnance Research under contract DA—11-022-ORD-1987* (January 15 1957); *J Electrochem. Soc* 104, 730 (1957)

4 M. Shaw and A. E. Remick, *J Electrochem. Soc.*, 97 324 (1950)

5. K. J Vetter *Z physik. Chem.*, 199, 300 (1952)

6. P J Hillson, *Trans. Faraday Soc.*, 50 385 (1954)

7 J E. Lilienfield and C. Miller *J Electrochem Soc.*, 100 222 (1953)

8. J O'M. Bockris, W Mehl, B. E. Conway and L. Young, J *Chem Phys.*, 25 777 (1956)

9. D C. Grahame *J Electrochem. Soc.* 99 370C (1952)

10. W Vielstich and P Delahay *J Am. Chem. Soc* 79 1874 (1957)

11 H. Gerischer and M. Krause, *Z physik. Chem. N F* 10, 264 (1957)

ON THE STRUCTURE AND DISTRIBUTION OF SPECIALIZED MUSCLE SYSTEM IN THE HEART OF BATS

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INTRODUCTION

A number of investigators (His, 1893 Tawara, 1906 Keith & Flack 1907 Davies, 1931 Davies & Francis, 1946) showed that in mammals the cardiac stimulus of contraction is initiated at and conducted by the sinuatrial node to the two atria From the atria the contraction impulses are picked up by the atrioventricular node and transmitted to an atrioventricular bundle which conveys this stimulus to the ventricles through its two limbs. Walls (1949 1945, 1947) Kistin (1949) and Prakash (1953 1954 1956, 1957) confirmed the presence of the sinuatrial and atrioventricular nodes and the atrioventricular bundle in the heart of mammals. The investigators quoted above also observed the muscular nature of the sinuatrial node the atrioventricular node and the bundle of His and favoured the myogenic initiation and conduction of the cardiac stimulus of contraction

The existence of a specialized muscular conduction system formed of nodal and Purkinje fibres has been questioned in mammals by Glomset (1941) Glomset & Glomset (1940 a, b) Glomset & Birge (1945 1948) and Glomset & Cross (1952) Glomset and his associates studied the heart of a large number of mammals but failed to find sinuatrial node, atrioventricula node atrioventricular bundle or any other specialized muscular connecting tissue Davies (1942) stated that parallel evolution of the conducting system has taken place in warm blooded animals and that this system is more extensive and better developed in birds than in mammals. To compare the nature and the distribution of nodal and Purkinje fibres (which constitute the impulse conducting tissue) of birds with that of mammals it has been felt desirable to study the heart and its conducting tissue in mammals which like bird are capable of active flight.

In the present investigation the heart and its conducting tissue has been studied in the bat, to ascertain the nature and the presence of sinuatrial node atrioventricular node and atrioventricular bundle.

MATERIAL AND METHODS

For the present investigation, commonly available bats have been selected. The hearts after being removed from partially chloroformed bats were fixed in Bouin s picroformol. Blocks were prepared according to paraffinembedding

method and serial sections 8-15 micra thick were cut. The sections were stained with acid fuchsin Pyridine silver method and Ungewitter s silver technique The structure of the fibres connecting the different chambers of the heart was studied with the help of 'Reichert s fibroscope Diagrams were also prepared through the help of this apparatus.

Observations

The heart of the bat resembles that of other mammals in having four chambers v iz. tw atria and two ventricles There is no sinus venosus. The right atrium is larger than the left and it extends over the latter s dorsal surface so as to cover it from above The two atria are separated by a prominent well developed interatrial septum. A well defined region representing the incorporated portion of the sinus venosus has been distinguished within the right atrium. The right atrium shows two portions one of the sinus venarum and the other of the proper right atrium

Sinuatrial Node (Keith Flack s node) An extensive well defined sinuatrial node is present in the upper part of the sulcus terminalis just near the opening of the right superior venacava into the right atrium The node is horse-shoe shaped forming a wide 'U' (Fig 1) The nuclei and the fibres forming the node take a deep stain with acid fuchsin so that the node is easily distinguished from the other tissue which lies around it. Pale multinucleated cells with abundance of nerve and muscle fibres are present. The nuclei of the cells also show great affinity for acid fuchsin and they lie surrounded by a clear space of cytoplasm (Fig 2) Some of the cells look empty as they do not have nuclei. The myofibrillae of the nodal fibres are sparse regularly placed and concentrated near the periphery of the fibres leaving a clear central area. In some of the cells very small granules have been observed in the clear space. The cells which have no nuclei in them have larger number of these granules. The nuclei of the node are more or less oval in shape. The other cardiac fibres which surround the node are comparatively larger in size and their nuclei tend to be more elongate and slender In transverse sections the nuclei of the nodal fibres are ringed by a few peripheral myofibrillae. The nodal fibres do not form compact bundles as does the cardiac muscle but tend to be more wavy and loosely arranged. No artery near sinuatrial node could be observed in bat though many other investigators (Walls 1942 Halpern 1955) previously recorded it in the heart of a few other mammals

The fibres from the cephalic end of the right limb of the 'U' shaped node extend towards the right atrium to become continuous with the atrial fibres A careful examination of the serial sections of the heart of the bat failed to trace any connection between the sinuatrial node and the atrioventricular node through Purkinje fibres or any other specialized tissue

Atrioventricular Node (Node of Tawara) The node of Tawara (Tawara 1906) is situated at the caudal end of the interatrial septum (Fig 3) It is

not well defined and is also not enclosed in a definite sheath and, therefore in transverse sections it does not appear as a definite structure. In serial sections it has been observed as a mass of loosely arranged Purkinje fibres and cells at the caudal extremity of the interatrial septum (Fig 4) The fibres and the cells forming this node communicate freely with the surrounding muscular component of the interatrial septum It is in the heart of the bat, that a mixing of the specialized fibres of the atrioventricular node with ordinary cardiac fibres, has been observed The absence of any specialized connecting tissue between the sinuatrial and the atrioventricular nodes and the fact that the gap between the two nodes is bridged through ordinary cardiac fibres indicate that in the heart of bats the cardiac stimulus of contraction initiated at the sinuatrial node would be conducted through ordinary cardiac muscle fibres to the atrioventricular node. The ordinary cardiac fibres and the specialized fibres (Purkinje fibres) present inside and between the sinuatrial and atrioventricular nodes form a continuum for the transmission of the impulse from the sinuatrial node to the atrioventricular node. A small nodal artery was observed near the node.

The structure and position of the cells, the fibres and the nuclei of the atrioventricular node resemble that described for them in the sinuatrial node. It was because of the loose arrangement of its cells and fibres that the atrioventricular node could be located in transverse sections The nuclei of the atrioventricular node also take a deep stain with acid fuchsin and its fibres which are comparatively thicker and show fewer striations intercross with each other to form cells. It is important to note that in the heart of bats the unique feature is the presence of a large number of muscle fibres extending from the caudal end of the atrioventricular node into the muscle component of the bundle of His which lies just beneath the atrioventricular node The presence of ordinary muscle fibres to connect the atrioventricular node with the atrioventricular bundle of the heart of bat indicates that the impulse conducting tissue is formed of both the histologically specialized and unspecialized tissue.

Atrioventricular Bundle (Bundle of His) The atrioventricular node is continuous ventrally through a number of ordinary muscle fibres, with the bundle of His (His, 1893) which lies in close association with the upper part of the ventricular septum The atrioventricular bundle is somewhat narrower and bent down a little towards the right side and comes to lie at the summit of the ventricular septum (Fig 5)

The bundle of His appears as a distinct structure in serial sections It is a compact mass of interwoven fine muscle fibres which take a deep stain with acid fuchsin indicating their histologically specialized nature. The bundle looks almost oval in transverse sections. The fibres of the bundle differ from other cardiac fibres primarily in their myofibrillar appearance The fibres of the bundle like the fibres of the sinuatrial node and the atrioventricular node

are distinctly broader containing fewer myofibrils. In some cases the myofibrils are limited to the periphery of the transitional fibres. On the whole the structure of the fibres of the bundle of His of the heart of these bats very much resembles with the Todd fibres (Todd 1932) recently sketched and described by Copenhaver & Truex (1952) in the heart of man, sheep and monkey.

A special feature of the heart of bat is the presence of numerous muscle fibres at the atrioventricular junction, in addition to the bundle of His. These multiple muscle fibres presumably act as additional pathways in addition to the bundle of His for transmitting the cardiac stimulus of contraction from atria to ventricles. In transverse sections the additional multiple atrioventricular muscular connections appear as a distinct bundle of muscle fibres which occupies a place very near to the atrioventricular bundle (Fig 5). The fibres of this additional bundle (bundle of Kent) (Kent, 1893) differ from those of the bundle of His. They are not interlaced with each other so as to form cells and do not have large oval deeply stained nuclei.

Caudally the bundle of His bifurcates into right and left limbs which descend down the respective sides of the interventricular septum (Fig 6). It is interesting to note that the heart of bat possesses like those of birds (Davies, 1930; Prakash, 1956a) a single muscular valve to guard the right atrioventricular orifice (Fig 7). Another special feature of great importance is that the right muscular valve receives an early branch from the right limb of the atrioventricular bundle to receive the contraction impulse much earlier than other portions of the ventricle. Such an arrangement helps the right muscular valve to contract at the outset of ventricular systole and consequently prevents regurgitation of blood into the right atrium. A similar condition has been described by Davies (1930) for birds.

Discussion

Glomset, Glomset and Birge (1944) did not believe in the presence of either nodes or bundles for impulse initiation and conduction in the heart of mammals. Glomset and Birge (1940) in their paper on the pathogenesis of heart block and bundle branch block deny the presence of the sinuatrial and atrioventricular nodes in the heart of mammals. They strongly challenged the myogenic initiation and conduction of the contraction impulse. The present study on the heart of the bat based on an examination of serial sections proves beyond doubt that the heart of mammals does possess a sinuatrial node, an atrioventricular node and an atrioventricular bundle to initiate, control and conduct the contraction impulses from atria to ventricles.

Davies (1930) observed that because of the rapid rate of heart beat in birds a quick transmission of the contraction impulse from the atria to the ventricles was necessary and for this purpose the avian heart possesses both the atrioventricular bundle and the multiple muscular connections. He also

stated that the conducting system in the heart of birds is better developed and more efficient than that of mammals, presumably because they possess accessory atrioventricular connections of Kent as well as the bundle of His. Prakash (1954a) pointed out that the mammalian heart which does not beat faster than that of avian does not need multiple atrioventricular connection of Kent (Kent, 1893) It is, therefore, clear that the birds possess accessory atrioventricular connection of Kent because they need them and mammals except for bats do not possess them because they do not require them. The presence or otherwise of any accessory atrioventricular muscular connections of Kent is correlated with functional requirements of the heart of individual birds and mammals

In spite of extensive research and detailed study of the heart of innumerable vertebrates there is no unanimity so far as the nature of the impulse initiating and conducting tissue is concerned. Davies and Francis (1946) in their review pointed out that a majority of investigators believe in the muscular nature of the impulse initiating and conducting tissue and favour the myogenic theory of cardiac conduction. In the present investigation it is observed that muscle fibres form an integral part of the impulse initiating and conducting structures namely the sinuatrial node the atrioventricular bundle and the Purkinje fibres Moreover the presence of a connecting muscle bundle (bundle of Kent) formed of only muscle fibres in addition to the bundle of His at the atrioventricular junction indicates the existence of a muscular pathway through which the atrial stimulus of contraction would travel to the ventricles. A continuity between sinuatrial and atrioventricular nodes and between the atrioventricular node and the atrioventricular bundle through unspecialized muscle fibres in the heart of bat shows that the existence of a muscular pathway can not be questioned and must be taken into account to "explain impulse formation and conduction in the heart of mammals"

There is no agreement even now between the various investigators with regard to the phylogeny of the impulse conducting tissue of the heart of birds and mammals. Davies (1930) described the atrioventricular muscular connections of birds as intermediate between those of fish and reptile on one hand and mammals on the other Davies & Francis (1941) regarded the nodal and Purkinje fibres of the hearts of birds and mammals as neomorphic in nature. Prakash (1953a,b, 1954c) denied the neomorphic nature of the impulse conducting system of the heart of birds and mammals The presence of accessory atrioventricular connections of Kent in addition to the bundle of His and the presence of a sinuatrial node without sinus venosus in the heart of bat indicates that evolution has taken place in the formation and development of the impulse conducting system of the heart of vertebrates.

In the heart of bat the cardiac ganglia are found only in relation to the atria. A large ganglion was observed near the anterior wall of the superior venacava. Two small ganglia were located in the wall of the left atrium and a large ganglion is present in front of the anterior wall of the left atrium. In

the caudal part of the interatrial septum islands of ganglion cells were observed and from here the nerve fibres extend into the atrioventricular node. The sinuatrial node is profusely innervated by post ganglionic fibres. A small ganglion is present in close vicinity of the sinuatrial node. Numerous nerve fibres could be traced into the substance of the atrioventricular node and all round it. No nerve cells and ganglia are present in the atrioventricular bundle and in the ventricles The ganglionic structures form definite groups around coronary sulcus. A network of subepicardial ganglionic plexus surrounds the posterior border of the interatrial septum. Groups of ganglion cells occur near the aortic roots Nerve fibres also accompany the larger blood vessels.

The nerve cells of all the ganglia are of the same type and the nerve ending shows gradual thinning leading to an abrupt termination

Summary

In the heart of bats the impulse initiating and conducting tissue is formed of sinuatrial node atrioventricular node atrioventricular bundle Purkinje fibres ordinary cardiac muscle fibres and multiple muscular connections of Kent. The hearts of bats possess multiple muscular connections of Kent in addition to the bundle of His for a quick transmission of the cardiac stimulus of contraction from atria to ventricles In this feature the heart of bat resembles that of birds and differ from that of mammals. A continuous muscular pathway exists to convey the cardiac stimulus of contraction from one chamber of the heart to the other The cardiac impulse initiating and conducting tissue of birds and mammals develops in accordance with the functional requirements of the heart of individual animals. The nervous component of the specialized muscle system as seen in the heart of bat has been described

Acknowledgements

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References

1 Copenhaver W.M. & Truex R. C. (1952) Anat. Rec. 114 (4) 601
2 Davies, F (1930) J Anat. Lond., 64 129
3 Davies, F (1931) J Anat. Lond., 65 339
4 Davies, F (1942) Brit. Heart J., 4 66.
5 Davies F & Francis E T B. (1941) Trans. Roy Soc Lond 231, B, 91
6. Davies, F & Francis, E. T B. (1946) : Nature. Lond 149 410
7 Davies, F & Francis E. T B. (1950) Biol Rev Cambridge 21 173.
8. Glomset, D. J & Glomset, A T A. (1946) : Biol Rev Cambridge 21 173.
9 Glomset, D J (1941) : Proc. Inst. Med. Chicago 13 393.
10. Glomset D. J & Glomset, A T A (1940a) Am. Heart. J 20 389.
11 Glomset, D J & Glomset, A T A (1940b) Am. Heart J 20 677
12. Glomset D J Glomset A T A & Birge R F (1941) : Am. Heart J 22 31

13. Glomset, D.J. & Birge, R.E. (1945) : Am. Heart J., 29 526.
14. Glomset, D. J. & Birge R.E. (1948) : Arch. Path. 45 135.
15. Glomset, D. J. & Cross K.R. (1952) : A. M. A. archives of Internal Medicine, 89 923.
16. Halpern, M. H. (1955) : Anat. Rec., 123 425.
17. His, W. (Jr) (1893) : Arb. Med. Klin. Lpz., 14
18. Keith, A. & Flack, M. W. (1907) : J. Anat. and Physiol., 41 172.
19. Kent, A. F. S. (1893) J. Physiol., 14 233.
20. Kistin A.D. (1949) : Am. Heart J. 37 (6) 849.
21. Prakash, R. (1953a) Proc. Zool. Soc. Bengal 6 (2) 113.
22. Prakash, R. (1953b) Proc. Rajasthan Acad. Sci. 4 1
23. Prakash, R. (1954a) Am. Heart J. 47 (2) 241
24. Prakash, R. (1954b) Ind. Med. J. July 164
25. Prakash R. (1954c) Proc. Zool. Soc. 7 (1) 27
26. Prakash, R. (1954d) Proc. Nat. Inst. Sci. India. 20 (6) 645
27. Prakash, R. (1956a) Proc. Nat. Inst. Sci. India. (B) 22 22.
28. Prakash, R. (1957) Indian Heart J. 9 (1) 1
29. Tawara S. (1906) Jena Gusta Fischer
30. Todd T. W. (1932) Cowdry' special Cytology 11 p. 1175
31. Walls E. W. (1942) : J. Anat. 76 (4) 359
32. Walls E. W. (1945) J. Anat. 79 45
33. Walls, E. W. (1947) : J. Anat. 81 (1) 93.

Abbreviations

AVB	Atrioventricular bundle.
AVN	Atrioventricular node.
IAS	Interatrial Septum
LA	Left Atrium
LAVV	Left Atrioventricular valve
LBB	Left Bundl Branch
LV	Left Ventricle.
MB	Muscle Bundle.
NA	Nodal artery
RA	Right Atrium
RBB	Right Bundle Branch.
SAN	Sinuatrial Node.
SAV	S nuatrial Valve
SVC	Superior Vena Cava.
V	Ventricle
VS	Ventricular septum.

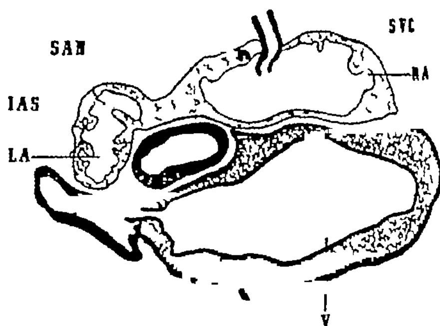


Fig No. 1 Diagram of transverse section of the heart of bat to show the position of sinuatrial node X 40

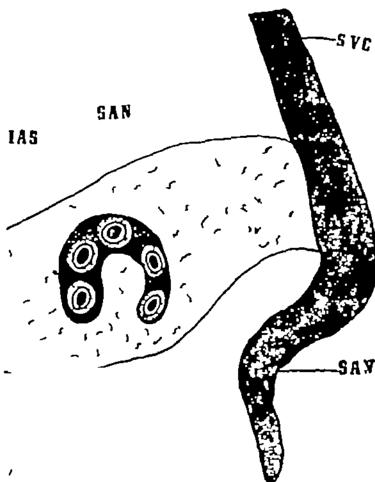


Fig. No. 2 Diagram showing the structure of the sinuatrial node × 350

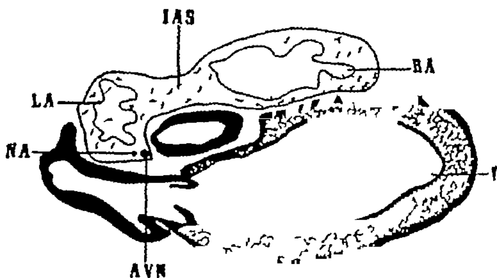


Fig. No 3 Transverse section of the heart of bat to show the atrioventricular node X 40.

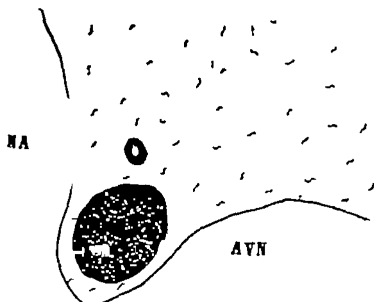


Fig No. 4 Diagram showing the structure of the atrioventricular node and nodal artery X 350

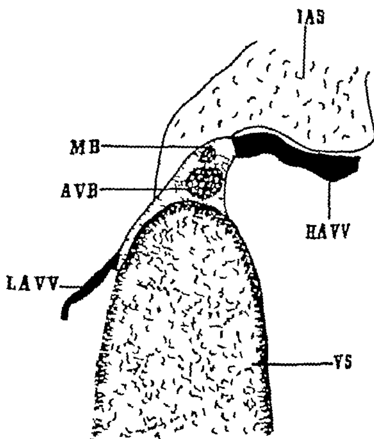


Fig No 5 Sagittal section to show atrioventricular bundle and accessory muscle bundle × 80

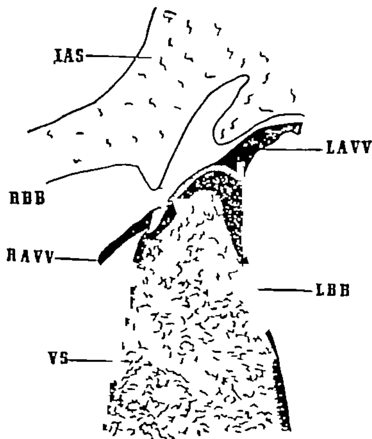


Fig. No. 6 Diagram showing th right and left branches of bundle of His in sagittal section. X 250

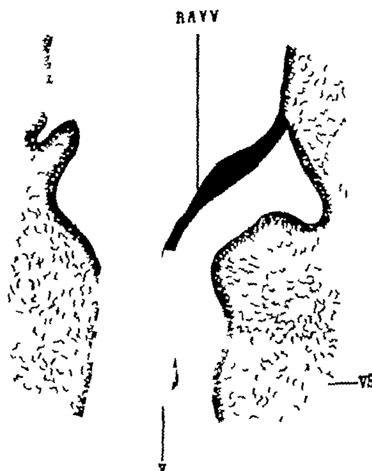


Fig No. 7 Diagram to show the muscular right atrioventricular valve X 20

OPTICAL ACTIVITY AND CHEMICAL CONSTITUTION

Part III—Physical Basis of optical activity

O N PERTI

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Drude (1900) obtained his formulae of rotatory dispersion by assuming the presence in the active molecules of oscillators with a definite period of vibration and these oscillators or electrons in order to respond differently to a *dextro* and *laevo* circularly polarized light were supposed to vibrate along a helicoid path. He obtained his formulae by suitably modifying the formulae of ordinary refraction as developed by Maxwell, Sellmeier Ketteler and Helmholtz. It has already been pointed out that Kuhn (1930) had shown the defect in the mathematical treatment of Drude indicating at the same time the validity of the formulae proposed by him. Even earlier than Kuhn Born (1918) criticised Drude s theory Speaking about optical activity he wrote 'The nature of these phenomena seemed to enforce the assumption that they must be bound up with a screw like structure of the molecules but such an assertion about the structure of the whole molecule has no place in ordinary dispersion theory which deals only with individual, quasi-electrically bound electrons or ions. It has therefore generally been customary to link up optical rotatory power with the electromagnetic theory of light only in a formal way in that new terms were included in the fundamental equations of the dispersion theory which expresses the screw-like, symmetryless character of the phenomenon, but which cannot be associated with any known forces. Drude appears to have been specially conscious of this avoidance of a deeper under standing of the molecular model, for he attempts, in his *Lehrbuch*, to make use of these additional terms more plausible through the hypothesis that electrons in the molecules are constrained to move in screw-like orbits. This conception, however seems to have found little support.

The theories of physical basis of optical activity that came after Drude are of significance only in that they were the fore runners of the very useful theory of Kuhn which had a great influence in this field. A brief account of these theories is gi en before Kuhn s theory is discussed.

Stark (1914a b) attempted to give a purely descriptive account of the origin of optical activity and suggested that optical rotatory power is produced not by a spiral vibrator but by the association of four dissimilar radicals with an asymmetric carbon atom. According to him when the electric vector of a light wave comes in contact with an electron t produces a displacement which is opposed by a restitutional fo ce acting in the direction of the chemical bond As a consequence of these displacing and restoring forces acting on the electron a displacement of t results which is not in general

parallel to the electric vector of wave field with the result that the polarisation of the molecule is slightly rotated from the direction of the electrical field. When random positions of the molecules are taken into account the rotation will be zero if the system contains one, two or three uncoupled valency electrons but four valency electrons if arranged dissymmetrically would lead to an over all definite rotation. The views of Stark were not followed here since it was no longer regarded essential that the restitutional forces acting on the valency electrons shall be anisotropic. A deeper understanding of the molecular model was proposed and the influence of one moving electron on the motion of others was taken to be the physical basis of the origin of optical activity

Oseen (1915) and Born (1915) proposed similar theories to explain optical activity Their fundamental suggestions were that like in the ordinary theory of dispersion we cannot neglect the dimensions of the molecules as compared to the wavelength of the incident light. Further the different resonators in a molecules are coupled' such that the displacement of one resonator influences the other Each unit of the molecule was treated as a charged particle which could be displaced slightly from its position of rest under the influence of an electrical field—a displacement which was opposed by a force whose components were given the form of linear functions of the components of displacement of all the particles. It was shown by Born that at least four non-planar coupled isotropic electrons are necessary to produce optical activity Like Stark s theory the theories of Oseen and Born confirmed the known relations between optical activity and molecular dissymmetry It may be mentioned that mathematical analysis on similar lines was also carried out by Lande (1918) and by Gans (1923 1924 1925 1926) In this theory the final formula for the rotation depends upon a large number of constants characteristic of the molecule, which it is difficult to link up with known data. The refractive power and the rotatory power in case of crystals of sodium chlorate and sodium bromate were calculated by Hermann (1923) and in case of β-quartz by Hylleraas (1927) but generally speaking this theory was not immediately useful so far as its applications to chemical problems is concerned. Born (19 0) himself later tried to simplify his formulae. In case of a system of four vibrators situated at the corners of a tetrahedron formed from four congruent triangles with identical lengths of sides Born s formula for the rotation is

$$\chi = 3\ 49 \times 10^{-11} \frac{\rho(n^2+2)}{M} f^2_{I}\ f^2_{II}\ \frac{\lambda_{I}\ \lambda^2_{II}}{(\lambda_{II}-\lambda_{I})^3}\left\{\frac{\lambda^2_{I}}{\lambda-\lambda^2_{I}}-\frac{\lambda^2_{II}}{\lambda-\lambda^2_{II}}\right\} \times$$

$$\frac{1}{l}\quad \frac{d}{l}\left(\frac{d^2}{l^2}-\frac{1}{3}\right)^2-\left(\frac{d^2}{l^2}+\frac{1}{3}\right)^{?}$$

where χ = rotation in degrees per dcm., ρ = density of the molecule, M = molecular weight n = refractive index, l = length in A. U of the base of

congruent triangles, d=perpendicular distance in A U between the middl of two edges of the tetrahedron λ_I, λ_{II} =characteristic wavelengths of two adjacent vibrators f_I f_{II}=strengths of the adjacent vibrators which are given by an expression of the type $\frac{e_I^2}{m_I}=f_I\frac{e^2}{m}$ $\frac{e_{II}^2}{m_{II}}=f_{II}\frac{e^2}{m}$ where e_I e_{II} m_I m_{II} are the charges and masses of the two vibrators and e m the charge and mass of an electron.

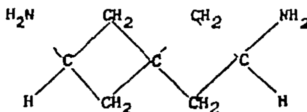
In order to apply this formula to a particular model Born makes the assumption $f_I=f_{II}=1$ and approximates the dispersion factor

$$\left\{\frac{\lambda^2_I}{\lambda^2-\lambda^2_I}-\frac{\lambda^2_{II}}{\lambda^2-\lambda^2_{II}}\right\} \text{ to } \frac{\lambda^2_I-\lambda^2_{II}}{\lambda^2}$$

when his formula reduces to

$$\lambda=3\ 49\times10^{-11}\ \frac{\rho(n^2+2)}{M\ \lambda^2}\left\{\frac{\lambda^2_I\quad\lambda^2_{II}}{\lambda^2_{II}-\lambda^2_I}\right\}^2\frac{1}{l^2}\ \frac{\frac{d}{l}\left(\frac{d^2}{l^2}-\frac{1}{4}\right)^2}{\left(\frac{d^2}{l^2}+\frac{1}{4}\right)^4}$$

This model of Born is almost identical with the spiro compound



the rotatory power of whose salt was found by Jansen and Pope (1932) to be $[M]_{5461}=16°$ From Born s approximate formula taking $l=1{\cdot}5$ A. U $d=4{\cdot}0$ A. U $\lambda_I=1200$ A.U., $\lambda_{II}=1600$ A.U., we get a value $[M]_{5461}=30°$ approximately Born s theory eliminates the use of Drude s highly artificial hypothesis of spiral v brators, instead t uses four isotropic vibrators correspond ing with the four dissymmetrically arranged radicals of an optically active compound. Born s general ideas were simplified by Kuhn who obtained a more practical f rmula by using only two linear vibrators at right angles to one another Before, however Kuhn s ideas are discussed a brief mention may also be made of other efforts in formulating physical basis of optical activity

Allen (1920) using Parsen s (1915) magneton concept of atomic structure developed a magneton theory of optical rotation. He replaced Drude s

electron moving in a spiral path by a magneton vibrating to and fro along a straight line and arrived at a formula where δ, the rotation per unit length, is given by the expression

$$\delta = \frac{2\pi^2}{\lambda^2} \Sigma \frac{\theta + k}{1 - \left(\frac{T_L}{T}\right)^2}$$

where L the number of magnetons in unit volume f = the strength of the absorption band, T and T_L are the frequencies of light and the natural frequency of the magneton respectively This equation is essentially similar to that given by Drude.

Thomsen (1920) in his paper entitled On Some Optical Effects Including Refraction and Rotation of the Plane of Polarisation Due to the Scattering of Light by Electrons gave a theory to account for the optical activity of dissymmetric molecules. His theory also assumes a coupling between resonators It further postulates that in an optically active molecule there are two dissymmetric systems one produced by a rigid tetrahedron of the four groups attached to the asymmetric carbon atom and the other corresponding to the valency electrons of the four bonds The valency electron dissymmetric system could be displaced by the action of the incident light but was opposed by a restoring force which tended to bring it back to its original configuration. With the help of this theory it was possible to calculate the magnitude of rotation and also to account for the occurrence of rotatory dispersion.

Mallemann (1925) considered a molecular model of an irregular tetrahedron whose edges are determined by the dimensions of the substituent radicals to each of which is attributed an electric vector Taking into account the existence of coupling forces between different resonators and the influence of the dimensions of the molecule he calculated the molecular rotation of CHClB I as $[M]_D \simeq 8°$ The compound however is not known and the result cannot be checked experimentally

Boys (1934) like de Mallemann tried to find a relation between the total optical rotation of an active molecule as a function of the refractivities of the groups and of their spatial distribution His model consists in having an isotropic medium in which the tetrahedra of four isotropic particles are distributed at random. When the electric field of a light wave comes in contact each atom becomes an oscillating electric doublet and these doublets cause secondary wavelets which when added together in the case of asymmetric model, change the plane of polarization of original wave The special rotation of a medium consisting of molecules containing one asymmetric carbon atom is given by an expression of the type

$$[\alpha] = 16{\cdot}62\,(n^2+2)(n^2+5)\,R_A\,R_B\,R_C\,R_D\,(1+\Gamma) \times \frac{(a-b)\,(a-c)\,(a-d)\,(b-c)\,(b-d)\,(c-d)}{\lambda^2\,M\,(a+b+c+d)^{11}}$$

Where M = molecular weight of the substance, n = refractive index of the medium, R_A, R_B, R_C, R_D = refractivities of the four groups A, B, C, D

a, b, c, d = effective radii of the groups A, B, C, D in A.U. and F is a function of the distances a, b, c, d. Boys theory like that of de Mallemann neglects the influence of the absorption bands unimportant in the consideration of the refractivity of a group. The drawbacks of these theories have been pointed out by Born. Both the refractivity and the rotatory power can be expressed

as sums of the terms of the type $\frac{a_i}{\nu_i^2-\nu^2}$. In case of refractive index the a_i

are all positive but in case of rotatory power their sum is zero so that some of them must be negative. It naturally follows that the two dispersions cannot be expressed identically.

Kuhn (1929, 1930[a,b]) has given a critical review of the fundamentals of the phenomena of optical activity. The more important points of the review are given below.

(i) Basing his arguments on Fresnel's equation for rotatory power namely $\phi = \frac{\pi}{\lambda\, n_0}(n_l - n_r)$ where ϕ is rotatory power and n_l and n_r are refractive indices for left and right circularly polarized light. Kuhn arrived at the conclusion: Even for substances of high rotatory power the difference between the refractive indices for left and right circularly polarized light is of the order of 1 part in 1 000 000.

(ii) Starting from the equations relating refractive index n of a medium for unpolarized light at wavelengths remote from the region of absorption with the position and intensity of absorption bands, namely

$$n^2 - 1 = \frac{L_0 e^2}{\pi m} \Sigma \frac{f_1}{\nu_1^2 - \nu^2} \qquad \text{for gases and}$$

$$\frac{n^2-1}{n^2+2} = \frac{L_0 e^2}{3\pi m} \Sigma \frac{f_1}{\nu_1^2 - \nu^2} \qquad \text{for liquids}$$

where L_0 = number of molecules per unit volume (Loschmidt number), e, m = charge and mass of the electron, ν_1 = frequency of the maximum selective absorption; f_1 = the strength of a given absorption band. (The value of f is related to the form of the band $f = \frac{mc}{\pi e^2} \cdot \frac{1}{L}\int \mu_\nu\, d\nu$ where $\int \mu_\nu\, d\nu$ represents the area of the absorption curve, being defined by the relation $\frac{I}{I_0} = e^{-\mu d}$) Kuhn arrives at the conclusion: "For most bands of active substances lying in the nearer ultraviolet the contribution to $(n_l - n_r)$ is in hundredths or thousandths of the contribution to the usual refractive index.

(*iii*) From (*i*) and (*ii*) it follows that if the total rotatory power of a compound in visible spectrum is the sum of the contributions of the inter bands in the Schumann region to the circular double refraction $(n_l \sim n_r)$ and if all were of the same sign and proportional to the intensity of the bands, the rotatory power would be several thousand times larger than any which has been observed Experimental results show however that the contribution of an optically active absorption band to $(n_l \sim n_r)$ is not proportional to the intensity of the absorption but is usually smaller for strong bands than for weak bands. Often it is the first weak absorption band which is found to govern the rotatory power in the visible spectrum. Kuhn, therefore, concluded 'On approaching shorter wavelengths, the rotation in general increases up to the point of passing through the first (weak) absorption band.

(*iv*) From (*i*) (*ii*) and (*iii*) he further concluded 'The relative difference in the behaviour towards right and left circularly polarised light in the strong bands lying in the outer ultraviolet $(f \sim 1)$ must often change sign in such a way that the rotation at a great distance from these bands disappears to a first approximation

From this summary of Kuhn's review it is seen that the rotatory power of a compound in the visible spectrum is chiefly governed by the nearest absorption bands situated in the visible and the near ultraviolet. The bands are probably of weak intensity as greater optical activity is associated with weak bands rather than with strong bands The intense absorption bands in the Schumann region are not of much consequence as partial rotations associated with them are not all of the same sign and hence cancel one another to a large extent. Optical rotatory power is physically equivalent to circular birefringence The optical rotatory power of a substance is physically equivalent to its property of having a different index of refraction n_r for right hand and n_l for left hand circularly poralized light. The rotation ρ produced by a layer 1 cm in length is related to the circular birefringence $(n_l - n_r)$ by the relation

$$\rho = \frac{\pi}{c} \nu \, (n_l - n_r)$$

where c = the velocity of light in vacuo and ν = the frequency of the light employed

Kuhn then worked out a special case of Born's theory of coupled electronic vibrators and developed formulae to express the circular dichroism and the rotatory dispersion inside an absorption band. In continuation of the original work by Born and by Oseen Kuhn also assumed that a positive or negative Cotton effect of a particular absorption band arises if the vibrating moment corresponding to an optical absorption band has non-parallel non-coplanar components in distant parts of the molecule The simplest oscillating system which would react differently towards *dextro* and *laevo*

circularly polarized wave would consist of two components which are a distance d apart and directed at right angles to each other and to the distance d. His model consists of two hormonic oscillators of charge e_1 and e_2 and mass m_1 and m_2 separated by a distance d along the Z-axis. In this model there are two anisotropic rectilinear oscillators One can oscillate only in the direction of OX and the other which is at a distance d from the first measured along OZ can oscillate only in the direction OY (Fig 1)

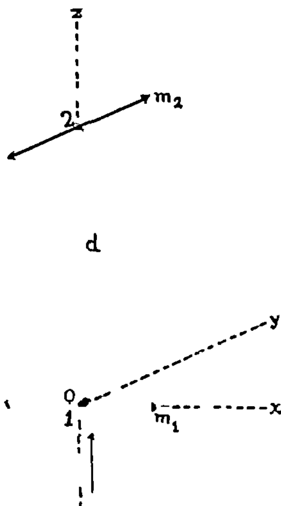


Fig 1

Kuhn's simplified model of a dissymmetric molecule

The charges e_1 and e and masses m_1 and m_2 of these particles are connected with e the charge and m the mass of an electron by the relations

$$\frac{e_1^2}{m_1} = f_1 \frac{e^2}{m} \qquad \frac{e_2}{m_2} = f \frac{e^2}{m}$$

where f_1 and f_2 represent the number of optical electrons per molecule. The f' value is a measure of the intensity of a band and is called the strength of the molecule.

In this particular system there would be no optical activity if the two particles vibrate quite independently of one another. In other words if there is no coupling force between them *dextro* or *laevo* circularly polarised light would have no preferential effect on the motions of the particles. If coupling force is introduced between the two particles the system would behave differently towards *dextro* or *laevo* circularly polarised light. Assuming very weak coupling Kuhn obtained the following expression for the rotation per cm for light differing widely in frequency of either vibrator

$$\phi = \text{Constant} \times \left\{ K_{1,2}\, d\, \frac{\nu^2}{\nu_1^2 - \nu_2^2} \left(\frac{1}{\nu_1^2 - \nu^2} - \frac{1}{\nu_2^2 - \nu^2} \right) \right\}$$

where ϕ = rotation $K_{1,2}$ = a constant characteristic of the coupling force between the oscillators, d = distance of separation ν = frequency of the incident light, ν_1 and ν_2 are the characteristic frequencies of the system. From the equation it follows that if $K_{1,2}=0$, then $\phi=0$ that is, if there is no coupling force between the electrons no optical activity would be there. Also if $d=0$ then $\phi=0$ which means that the two electrons must be separated by a finite distance otherwise there would be no optical activity. This equation involves two terms of opposite sign and indicates that partial rotations due to the two coupled electrons will be opposite in sign if $\nu < \nu_1$ and $< \nu_2$, that is, at frequencies which are less than that of either absorption band. Since the sum of two numerators is zero the partial rotations due to two coupled electrons will tend to cancel one another at longer wavelengths unless their frequencies are widely separated. Here it may also be pointed out that the magnitude of the coupling between the two resonators depends upon the relative intensities of the absorption bands associated with the two electrons. A strong coupling will be needed if both give rise to strong absorption bands but a weak coupling will suffice if one of the bands is strong and the other weak.

Kuhn applied his formula in cases of molecules where conditions postulated by him are somewhat obtainable. The compounds investigated were axially symmetrical molecules such as dipyruvic erythritol a symmetrical spirane investigated earlier by Boeseken and Felix (1928) and symmetrical cobalt complexes $K_3[Co\ 3C_2H_4]$ and $[Co\ 3C_2H_4(NH_2)_2]Br_3$ (Kuhn, 1931 b). However it is not in the realm of configuration determination that the practical utility of Kuhn's views lie.

Kuhn also arrived at expressions for optical rotation and circular dichroism which were identical with those of Drude if the various constants in Kuhn's equations are included in the constant D of Drude's equation (see earlier section giving Drude's formula in the form expressed by Natanson and

Bruhat) He also showed that the constant D could be expressed as a linear function of a factor g' called the anisotropic factor If a new group with a definite absorption band is introduced into an active molecule it becomes anisotropic if it is coupled. Kuhn calls it 'induced anisotropy The anisotropic vibrations of this band influence the 'induced anisotropies' of the vibration of other absorption bands with which it is coupled and we get an effect which is termed by him as 'vicinal function It follows that the coupled absorption bands of each substituent play a two-fold role in the total molecular rotation. In the present state of our knowledge it is not possible to predict the vicinal effect of any given arrangement of substituents since only in a very few simple cases absolute configurations are known and generally all the information available in relation to optical activity and chemical structure has so far only been obtained by emp rical methods.

Kuhn has recently given (Kuhn 1958) a simplified formula for molecular rotation in degrees and has also pointed out the physical significance of the 'anisotropic factor For molecular rotation expressed in degrees

$$\left[M\right]^{\circ}_{\nu}=2\cdot1\times10^{3}\frac{n^{2}_{\nu}+2}{3}\nu^{2}\int_{\nu_1=0}^{\infty}\frac{1}{\nu_1}\frac{3}{n^{2}_{\nu}+2}(k_1-k)\frac{d\nu_1}{\nu_1^{2}-\nu^{2}}$$

where n_ν and n_ν represent the index of refraction for light of frequency ν viz., ν_1 of the medium which may be a gas, a pure liquid, or a solution of the optically active substance The $\left[M\right]^{\circ}_{\nu}$ given in the equation is one hundredth of the value of specific rotation multiplied by molecular weight. k_1 and k in the equation are the molecular absorption coefficients of the optically active substance for left and right hand circularly polarized light of frequency ν_1 The molecular absorption coefficient used here is defined by the statement that the intensity of a beam of *dextro* circularly polarized light of frequency ν will on passing through x cm of a solution of concentration C gm mol/litre, decrease from J to J $J=J_0\ 10^{-k\cdot c\cdot x}$ The integral in the equation has to be taken over all values of ν_1 He has also pointed out that k, the absorption coefficient of the racemic substance for ordinary light is related to circular

dichroism $k=\frac{k_1+k}{2}$

and g' the anisotropy factor is approximately equal to the relative difference of the absorption coefficients for left and right hand circularly polarized light, $\frac{k-k}{2}=g$, and depends, in general on the frequency ν The physical significance of the anisotropic factor can be seen from Fig 2 indicating th two vibrating systems. In the left hand part of Fig 2-A and 2 B is shown the simplest mode of vibration of two components which are a distance d apart and directed at right angles to each other and to the distance d The effect

where f_1 and f_2 represent the number of optical electrons per molecule. The f value is a measure of the intensity of a band and is called the oscillator strength of the molecule.

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absorption the equation of Kuhn and Braun was tested by them in the case of methyl-α-azido propionate, α-azido propionic dimethyl amide and ethyl-α-bromo propionate. In case of α bromo propionic dimethylamide the measurements could not be extended far into the region covered by the absorption band on account of its great intensity Kuhn Freudenberg and Wolf (Kuhn, 1930a) investigated methyl-α-chloro propionate and α-chloro propionic dimethylamide Kuhn and Lehman (Kuhn 1931 1932) investigated in detail the anomalous rotatory dispersion of β-octyl nitrite a compound prepared and studied previously by Pickard and Hunter (1923) In these investigations there was an approximate agreement between the theoretical and experimental curves.

Lowry and Hudson (Lowry *et al.* 1933) found that the experimental equation representing the absorption curve fits the experimental data better if λ, the wavelength, is the variable instead of the frequency ν The equation using λ as variable was applied by these workers in the case of ethyl -(+)-bornyl xanthate, diphenyl-(−)-menthyl dithiourethane (−)- menthyl dixanthate, tetra-acetyl−μ−arabinose and penta-acetyl−μ−fructose Since absorption bands are, as a rule symmetrical on a scale of wavelengths and not on a scale of frequencies the experimental results fit better with Lowry's equation.

The rotatory dispersion equation in the region of transparency away from the absorption bands is usually expressed as containing several terms in summation

$$[\alpha]_{\lambda}^{t} = \frac{K}{\lambda^2 - \lambda'^2} + \frac{K'}{\lambda^2 - \lambda''^2_0} + \frac{K''}{\lambda - \lambda} + \ldots$$

where λ_0, λ'_0, λ''_0 ... etc. are the characteristic wavelengths. In practice, however the rotatory dispersion curves can be usually expressed by one or two terms in the dispersion equation (Lowry 1924) Based on the one electron theory (See Condon Altar and Eyring 1937 Condon 1937 Gorin, Walter Eyring 1938 Kauzmann Walter Eyring, 1940 Kirkwood, 1937 1939) which encompasses the fact that the vibrating moment according to wavemechanics is, even, in the first approximation, distributed over the entire region in which the ψ-function corresponding to the electron in question, is different from zero. Condon has suggested that these Drude terms be considered as having average values resulting from the combination of several terms with more fundamental significance Kuhn analysis clearly shows that the contribution of coupling to optical activity will dominate in most cases (Kuhn, 1952) The one electron contribution formally corresponds to higher approximation terms only of the coupling effect and only in special cases, presumably with very weak absorption bands there may be exceptions where the one electron contribution will reach a magnitude comparable to the value of the coupling effect. It follows that the absorption bands which are important for refraction are insignificant in optical activity Kuhn (1958)

has drawn attention to the high value of 'g' the anisotropy factor in the case of weak absorption bands. Its consequence is that the rotatory contribution of weak bands in the visible is as important as the contribution of strong bands especially where weak absorption bands are situated in the near, and strong bands in the far ultraviolet. The importance of weak bands is enhanced by an extensive mutual cancellation of the far ultraviolet contribution. At the same time the weak absorption bands must be extremely sensitive as far as their rotatory contribution is concerned while strong absorption bands are less alterable. This incidentally provides a better understanding of the frequently encountered high sensitivity of optical rotatory power to chemical or physical changes.

Not much work has been reported in the literature on the direct experimental correlation between 'characteristic' wavelengths and the absorption spectra of optically active compounds. There is, however some evidence to indicate that the characteristic wavelengths ($(\lambda_0 s)$) deduced from rotatory dispersion equations in the visible region are in agreement with those obtained by direct measurement. Thus the 'characteristic' wavelength deduced from rotatory dispersion equation of camphorquinone in 1% benzene solution at 35° is 4690 A.U (Singh *et al* 1931) and in 15% benzene solution at 20° is 4730 A.U (Lowry *et al*., 1925) which compares favourably with the values of absorption maxima in benzene, namely 4700 A U as reported by Lowry and French (Lowry *et al*., 1924) The ultraviolet absorption of camphor was also studied by Lowry and French and the value of one of the absorption maxima obtained by them has been found by Singh and Nayar (Singh *et al*., 1947, 1948) to be in close agreement with the longer 'characteristic' wavelength deduced from the rotatory dispersion equation

$$[\alpha]^{35°}_{\lambda} = \frac{21.91}{\lambda^2 - 0.087} - \frac{11.5}{\lambda^2 - 0.037}$$

The equation gives $\lambda_0 = 2950$ A.U and $\lambda_0 = 1924$ A.U The 'characteristic' wavelength 2950 A.U corresponds to the known weak absorption band of the carbonyl group The other characteristic wavelength of 1924 A.U probably represents the weighted average of many weak and strong bands farther in the ultraviolet. Pickard and Hunter (1930) found λ_0 from the rotatory dispersion and λ_{max} from absorption spectra of (+)−γ−nonyl nitrite to be almost identical (λ_0=3680 A.U and λ_m =3670−3778 A.U.).

Singh and Amma (Singh *et al*., 1957) studied the ultraviolet absorption spectra of active (+)−camphor-β-sulphonyl phenylamide (+)−camphor-β-sulphonyl-o-(*m* and *p*-)-bromo phenylamide, methylamine-(+)-camphor-β-sulphonate and 2-N-(methyl-ketimine)-(+)-camphor 10 sulphonic acid In all these cases the rotatory dispersion in the visible region can be expressed by Drude's one term equation and consequently one 'characteristic' wavelength can be calculated for each compound. Direct absorption measurement is found to give more than one absorption maxima for each compound. It was

found that the 'characteristic' wavelength is almost identical with one of these absorption maxima or the average value of the different absorption bands. These studies were extended by the study of optically active salts of Reychler's acid with *o*- *m*- and *p*- chloro and bromo anilines (+)-camphor-β-sulphonyl-*o* *m*- and *p*- chloro and bromo phenylamides and (+)-camphor-β-sulphonamide and its anhydramide (Singh *et al.*, 195) It was noted that there is a good agreement between one of the absorption maxima λ_m in the near ultraviolet and the characteristic wavelength λ_0 derived from Drude's one term equation. Singh and Saxena (Singh *et al* 1958) studied ultraviolet absorption spectra of active *p*-sulphonamido- *o*- *m*- and *p*-methoxy phenylimino camphors. The rotatory dispersion of these compounds is simple and in the visible range it can be expressed by Drude's one term equation Thus in each case a single characteristic wavelength can be obtained The ultraviolet absorption spectra of these compounds generally show two absorption maxima and the one near the 'characteristic' wavelength is found to differ widely from it. The difference between λ_0 and λ_{max} range from 0·2 mμ to 83 7 mμ, it being more in the arylimine than in the arylamine compounds. The authors have suggested that the discrepancy is probably due to the experimental limitations of the method used in determining optical rotatory power and the dispersion equation used by them only approximately represents the distribution of rotation over a very small range of wavelengths Singh and Verma (Singh *et al.*, 1958[b]) found similar type of results in the case of active camphor-β-sulphonyl-methoxy (*o*- *m*- and *p*-) and ethoxy (*m*-and *p*-) phenylamide in methyl alcohol. Singh and Saxena (Singh *et al* 1960) studied the absorption spectra of (+)-camphanoquinoxaline, *m*- and *p*- phenylene bis-imino-(+)-camphor *m*- and *p*-phenylene bis-amino-(+)-camphor and (+)-camphanodihydro-quinoxaline They found that the rotatory dispersion of these compounds in the visible range can be expressed either by one term equation or two term equation. The 'characteristic' wavelengths derived from either equation do not agree with the observed ultraviolet absorption maxima These 'characteristic' wavelengths represent only the weighted average of the observed bands and those unobserved in the far ultraviolet.

References

1 Allen, *Phil. Mag* 40 426 (1920)
2 Born *et al.*, *Phy Zeit.*, 16, 251 (1915) ; *Ann. Physik.*, 55 177 (1918) ; *Elementare Quantenmechanik* (1930)
3 Boeklin & Felix., *Ber* 58 1855 (1928)
4 Boys, *Proc. Roy Soc* 144A 655 (1934)
5 Condon, *Rev. Mod. Phys* 9 433 (1937)
6 Condon, Altar & Eyring, *J Chem. Phys* 5 753 (1937)
7 de Mallemann, *Compt. rend.*, 181 298 (1925).
8 Drude, *Theory of Optics* (1900)
9 Gans, *Z. Physik.*, 17 353 (1923) ; 27 64 (1925) ; *Ann. der Physik.*, 79 548 (1926)
10 Gorin, Walter & Eyring, *J Chem. Phys.*, 6 824 (1938)
11 Hermann, *Zeit. f Physik.*, 16 103 (1923)

12 Hylleraas *Zeit. f. Physik* 44 871 (1927)

13 Jansen & Pope, *Chem. & Ind.*, 51 316 (1932)

14 Kauzmann Walter & Eyring, *Chem. Rev* 26, 339 (1940)

15 Kirkwood *J Chem. Phys.*, 5 479 (1937) 7 139 (1939).

16 Kuhn *et. al.*, *Z. Ph. C.* B4, 14 (1929) B8 281 (1930) *Trans. Farad. Soc* 26 293 (1930) *Ber.*, 63 190, 2367 (1930c) *Z. Electrochem.* 37 549 (1931) *Z. Ph. C.*, B20, 325 (1932) ; *Drehung der Polarisationsebene des Lichts Hand und J Wien der dem 1 Physik* 8 Leipzig (1932) ; *Ber* 20B 325 (1933) *Z. Ph. C.*, B24 335 (1934) *Z. anorg. Chem.*, 216 321 (1934b) ; *Z. Electrochem.*, 56, 506 (1952) ; *Am. Rev. Phys. Chem.*, 9 417 (1958)

17 Lande *Ann. der Physik.*, 56 225 (1918)

18 Levine & Rothen *J Chem Phys* 2 681 (1924)

19 Lowry *et. al.*, *J Chem Soc* 125, 1593, 1971 (1924) ; 127 607 (1925) *Trans. Farad. Soc.*, 232A, 117 (1933)

20 Natanson, *Bull. Acad Sci. Krakow* 764 (1908) *J de Phys.* (iv), 8 321 (1909)

21 Oseen *Ann. Physik* 48 1 (1915)

22 Paschen *Smithsonian Collection* No. 2371 (1915)

23 Pickard & Hunter *J Chem. Soc.*, 123 434 (1923)

24 Singh *et. al.*, *Jour Ind. Chem. Soc* 8, 35 (1931) ; *Proc Ind. Acad. Sci.* 26A, 203 (1947) 27A, 67 (1948) *J Sci. & Ind Res.*, 16B 202 486b (1957) ; 17B, 181 (1958) ; *J. Ind. Chem. Soc.* 35, 227 (1958b) ; 37 273 (1960)

25 Stark, *Jahrb Radioakt.*, 11 194 (1914a) ; *Principien der Atomdynamik*, 3, 342 (1915)

26 Thomson, *Phil Mag* 40, 713 (1920)

EFFECT OF TRACE ELEMENTS (II) ON NITROGEN FIXATION

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Bortels (1937 & 40) reported that molybdenum was required by *A chroococcum* for growth and nitrogen fixation Vanniel (1935) Konishi and Tsuge (1933) found that the addition of molybdenum and vanadium to the soil may increase bacterial number and nitrogen fixation. Burk (1934) studied the effect of varying concentrations of molybdenum and vanadium on nitrogen fixation and observed that molybdenum, replaceable by vanadium is specific for nitrogen fixation. Jensen (1943) on the other hand reported that *A indicum* requires molybdenum for nitrogen fixation, but could not be replaced by vanadium Steinberg (1945) Jensen and Spencer (1946) Mulder (1948) have reported the requirement of molybdenum by *A niger* and other nitrogen fixing bacteria

Brenchley and Thornton (1925) reported that growth of *A chroococcum* was optimum in soil and culture solution of pH 6.8 at a concentration of 0.5 ppm boric acid. Maze (1914) and Warrington (1923) found Boron and Zinc essential for normal growth of Azotobacter Brenchley (1943) reported poor development of nodule bacteria on roots of legumes that were boron deficient. Eyster (1952) Yogeswari (1948) Herzinger (1940) Anderson (1950) and Gerretson (1953) have reported boron essential for various fungi algae aerobic soil bacteria and nitrifying bacteria and azotobacter

Lathan (1909) and Mulder (1948) have found stimulating effect of Zinc on growth of algae and leguminous bacteria.

Lees (1946) and Swanback (1950) noted a timulating effect of copper on ammonifying, nitrifying, and nitrogen fixing organisms Bortels (1937) established the requirement of copper for normal growth of *A niger*

Gregario (1916) Kayser (1921) Kayser and Delaval (1924-25) and Rocasolana (1938) have observed that manganese accelera es nitrogen fixation. Preliminary results obtained by Heintz and Mann (1949) showed that role of oxidation of manganese ions may be reduced by some copper enzymes and Gerretson (1937) demonstrated manganese precipitation by various micro-organisms contained in soils.

In addition to these, behaviour of a number of other elements on soil micro-organisms have also been studied. Kaserer (1911) Reny and Rosing (1911) Sohngen (1913) Blom (1931) Burk and Lineweaver (1931) Burk (1932) and

Horner and Burk (1934) have reported stimutive effect of iron and calcium on nitrogen fixation by the soil micro-organisms. Recently Iswaran and Sundara Rao (1960) have studied the behaviour of Mo, W U Cu, Mn Fe, Co, Ni and Pb and observed the stimulative effect of Mo, Co Ni and Fe (k) on nitrogen fixation by *Azotobacter* They further produced a definite evidence of absorption of Mo and Co in the body cell of Azotobacter

EXPERIMENTAL

The experiment, in four replications, was conducted in "Ashby's (Fred & Waksman 1928) liquid culture medium and in soil as well. In 250 c.c. of the medium 0 1 ppm. and 0.2 ppm. trace elements was added. After inoculating the medium with 1 c.c., of azotobacter culture previously isolated and cultivated the flasks were incubated at 30° C After a period of 7 14 and 21 days incubation, flasks were taken out and analysed for organic carbon and total nitrogen.

Experiment in soil was conducted with 150 gms of well powdered and seived field soil To the soil 25 c.c. of Ashby's liquid culture medium and 0.1 ppm. and 0.2 ppm. of the trace elements were added. After mixing them well, they were incubated at 30° C. The content of the flask was stirred daily to facilitate aeration. After a period of 7 14 and 21 days samples were drawn out and analysed for organic carbon total nitrogen and nitrate nitrogen.

Organic carbon was estimated by Walkley and Black's (1934) method total nitrogen was determined by the standared Kjeldhal method as modified by Bal (1925) and nitrate nitrogen was determined by Harper's (19?) phenoldisulphonic acid method.

RESULTS AND DISCUSSION

From the data in table no 1 it is observed that trace elements have their definite role on the fixation of atmospheric nitrogen. Trace elements treated culture solution at both the concentrations fixed more nitrogen than the control at the different dates of analysis There was more of oxidation of organic carbon in the trace elements treated culture than in the control. Copper Zinc, Boron and Molybdenum at both the concentrations gave better results than the others. Cobalt induced lowest nitrogen fixation where as Molybdenum stimulated highest nitrogen fixation This corroborates the finding of Vanneil (1935) Bortels (1937) Mulder (1918) Steinberg (1915) Dhar and Nagpal (1956) and Iswaran and Sundara Rao (1960) It is also noted that higher dose of concentration fixed more nitrogen and oxidised more organic carbon than the lower but the values of C/N ratio indicate that the oxidation of organic carbon and the fixation of atmospheric nitrogen are not proportionate to each other as the C/N ratios for the higher dose of concentration of trace elements are narrower than for the lower This suggests that 0.2 ppm. concentration of trace elements are economical than 0 1 ppm. concentration.

TABLE 1

Total nitrogen fixed in 100 c of per culture medium.

Organic carbon at start 0.360 gms. %

Treatments		7 days				14 days				21 days			
		Organic carbon in the end in gms	Carbon oxidised in gms	Nitrogen fixed in gms	C/N ratio	Organic carbon in the end in gms	Carbon oxidised in gms	Nitrogen fixed in gms	C/N ratio	Organic carbon in the end in gms	Carbon oxidised in gms	Nitrogen fixed in gms	C/N ratio
Control		0.350	0.010	0.0012	292	0.340	0.020	0.0022	155	0.320	0.040	0.0042	74
Cobalt	0.1 ppm	0.325	0.035	0.0045	72	0.315	0.045	0.0062	51	0.290	0.070	0.0102	28
	0.2 ppm	0.310	0.050	0.0068	46	0.305	0.055	0.0078	39	0.290	0.070	0.0100	29
Manganese	0.1 ppm	0.315	0.045	0.0060	53	0.303	0.055	0.0078	39	0.290	0.070	0.0100	29
	0.2 ppm	0.300	0.060	0.0084	36	0.290	0.070	0.0102	28	0.278	0.083	0.0122	23
Copper	0.1 ppm	0.305	0.055	0.0076	40	0.295	0.065	0.0091	32	0.280	0.080	0.0111	25
	0.2 ppm	0.290	0.070	0.0103	28	0.280	0.080	0.0124	23	0.265	0.095	0.0154	17
Zinc	0.1 ppm	0.300	0.060	0.0085	35	0.290	0.070	0.0101	29	0.275	0.085	0.0128	22
	0.2 ppm	0.285	0.075	0.0115	25	0.275	0.085	0.0132	21	0.55	0.105	0.0171	15
Boron	0.1 ppm	0.290	0.070	0.0104	28	0.285	0.075	0.0113	25	0.255	0.105	0.0168	15
	0.2 ppm	0.275	0.085	0.0134	21	0.270	0.090	0.0143	19	0.250	0.110	0.0179	14
Molybdenum	0.1 ppm	0.285	0.075	0.0113	25	0.270	0.090	0.0147	18	0.245	0.115	0.0195	13
	0.2 ppm	0.270	0.090	0.0143	19	0.258	0.105	0.0175	15	0.243	0.118	0.0204	12
C D t 1%				0.0019			...	0.0025	..		...	0.0031	..

[illegible]ium	0.8 ppm	[illegible]					
Molybdenum	0.1 ppm	150.7	227.0	192.0	183.5	270.0	280.0
	0.2 ppm	158.6	256.0	193.0	175.5	511.1	666.6

In view of the fact that organisms live and act in association in soil and that the important information about their activities is obtained in associated soil culture, the experiment was conducted in soil as well. Data in table 2 indicate that there was greater fixation of nitrogen in the soil than in the culture medium. It is also noted that oxidation of organic carbon was also higher in soil than in the culture medium. This might be due to the beneficial action associated by other organisms in the soil. This also corroborates the findings of Beijrinck (1925) Molybdenum seems to stimulate nitrogen fixation more than the other trace elements.

Decrease in the values of nitrate nitrogen at 7th day over the values of nitrate nitrogen at start indicates an active bacterial development, which lowers down the nitrate content of the soil. It was also noted that greatest utilization of nitrate was in molybdenum treated soil which also showed maximum nitrogen fixation and the lowest in cobalt treated soil which showed the lowest nitrogen fixation. It is further observed that there is an increase in the values of nitrate nitrogen on 14th and 21st day This indicates that nitrifying organisms present in the soil play their role simultaneously After deducting the values of carbon oxidised and nitrogen fixed at 7th day from their corresponding values at 14th day and the values at 14th day from their corresponding values at 21st day both in liquid and soil (table 1 and 2), the efficiency of the nitrogen fixing organism at different intervals was calculated. These values are incorporated in table 3

A perusal of the data in table 3 reveals that the trace elements under study increased the efficiency of the nitrogen fixing organisms. Younger cultures in liquid culture medium appear to fix more nitrogen than the older ones, probably due to the fact they are more active when young and also due to the waste toxic product formed in the older culture by the activities of the organism which inhibits their further growth and activity This falls in line with the observation of Pathak and Shrikhande (1953)

When compared for the efficiency of organisms in pure culture and soil culture, it is observed that the organisms in soil are more efficient than the isolated organisms in the pure culture. In soil the organisms are rather more efficient at 21st day than at 7th and 14th day

This perhaps is due to the benefit derived from the action of the associated organisms in the soil as pointed out by Waksman (1927) This was first recognized by Beijrinck (1925) who even went so far as to suggest that Azotobacter when grown in pure culture was incapable of fixing any appreciable amount of nitrogen, but that large amount of nitrogen was fixed in presence of other organisms. Waksman (1927) has emphasized that the presence of other organism is advantageous to nitrogen fixation The other organisms either eat the waste products or create otherwise favourable conditions. This observation also finds a good support by the work of Richard (19[illegible]) who has demonstrated that organisms work more efficiently in association

SUMMARY

Effect of some trace elements viz., Molybdenum Boron Zinc Copper Manganese and Cobalt on nitrogen fixation was studied in liquid culture medium and in the soil culture medium at 0 1 ppm. and 0.2 ppm. concentrations. It was observed that

1 Molybdenum treated culture fixed highest nitrogen at both the concentrations.

2. Yonger culture fixed more nitrogen than the older ones and they were more efficient than the older ones.

3. Amount of nitrogen fixed in the soil culture was more than the amount of nitrogen fixed in pure culture, and also the organisms in soil were rather more efficient.

4. There was utilization of the No_3 N initially present in the soil culture medium during first 7th day of analysis. Later on there was a tendency to increase the nitrate contents which was more pronounced in Molybdenum treated soil.

5. There was decrease in C/N ratio of the liquid culture as well as in soil culture with the advancement of period of decomposition.

REFERENCES

1 Anderson F G. (1950) Trace elements in plants and animals by Stiles, W (1946)
2. Ball, D.V (1925) Jour Agri Sci. 15; 454
3. Beijrinck, N W (1925) Central Bakt. II. 63 353-359
4. Elson, J (1931) Zbl. Bakt 84 325.
5 Bortels, H (1937) ; Arch. Mikrobiol. 8 13-26.
6 Bortels, H. (1940) ; Arch Mikrobiol 11; 155-286.
7 Brenchley W.B. (1943) ; Bio. Rev Camb. Phil. Sco. 18 159-171
8. Brenchley W.B. and Thornton H. G (1925) Proc. Roy Sco (Lond.) B. 98:373-399
9. Burk, D (1932) Proc I ter Cong Soil Scien. 3; 67
10. Burk, D (1934) ; Ergebnisseder Enzymfors Chung 3 23
11 Burk, D and Linweaver (1931) Arch Mikrobiol 2; 155
12. Dhar N R and Nagpal, K L Proc Nat Acad Scie India 25 pt 3rd. Sec. A (1956)
13. Eyster C. (1952) Nature 170; 755
14. Fred, E B and Waksman, S A. (1928) Lab. Manual of General Microbiology Ist Ed. (New York.)
15. Gerretsen, F C (1937) ; Ann Botany (N.S) 1; 207-230
16. Gerretsen, F C (1955) 6th Inter Cong Mikrob Rome. Abst Commins 3; 120-222.
17 Gregario R.A. (1916) Bul. Agri. Intern. 129 510.
18. Harper H.H. (1924) Ind. Eng. Chem. 16; 180
19. Heintz, S. G. and Mann P J G (1949) Jour Agri. Sci. 39,80-95.
20 Herzinger F (1940) Bodenk. U.P flanzenerhahr 16 141 168.
21. Horner C.K. and Burk, D (1934) Jour Agri. Res. 48 981-995
22. Iswaran, V and Sundara Rao, W V B. (1960) Proc. Ind. Acad. Sci. 51 103.
23. Jenson, H. L. (1943) 3rd. Proc. Linnean. Soo (N S. wales) 68; 1-8

24 Jensen, H. L. and Spencer D (1946) : Aust. J Sci. 9; 28.
25. Kaserer H (1911) : Zbl. Bakt. 84; 325.
26. Kayser E. (1921) : Comt. Rend. 172 183 491 939 1113.
27 Kayser E. and Delaval, H. (1924) : Compt. Rend 179; 11
28. Kayser E. and Delaval H. (1925) : Compt. Rend. 181; 151
29 Konishi, K. and Tsuges, T (1935) : Jour Agri. Soc. (Japan) 8.
30. Lathan M.E. (1909) : J Agric. Res 6 407
31 Lees, H (1946) : Nature. 158; 97
32. Maze P (1914) : Trace elements in plants and animals by Stiles, W (1946) 42
33. Mulder E. G (1948) : Plant and soil, 1; 94-119.
34 Pathak, A.N and Shrikhande J G (1953) : Jour Indian Soc. Soil Sci. Vol. 1 : 111.
35 Remy T and Rosing, G (1911) : Zbl. Bakt. 43; 168.
36. Richard, E H. (1935) : Jour Agri. Sci. 25; 296.
37 Rocasolana, R. A De G (1938) : Expt. Stn. Rec. 38 : 122.
38. Sohngen, N L. (1913) : Zbl. Bakt 38: 621
39 Steinberg, R. A. (1945) : Soil Sci. 60; July Dec.
40 Swanback, T R. (1950) : Agri. Expt. Station Bull. 535.
41 Vanniel, C B (1935) : Arch. Mikrob. 6: 215-218.
42. Waksman S.A. (1927) : Principles of Microbiology
43 Walkley A. and Black I A (1934) Soil Sci. 37 29
44 Warrington, K. (1923) : Trace elements in plants & animals by Stiles, W (1946); 1
45. Yogerwari, L. (1946) : Int. Proc. Ind Acad Sci B 23; 177-201

ANALYTICAL STUDIES OF SOME INDIAN CERAMIC CLAYS AND THEIR BASE EXCHANGE PROPERTIES

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On the basis of the generalisations of Pauling (1930) the atomic structures of the common clay minerals have been determined in considerable detail by numerous investigators. The clay minerals have been broadly classified on their structural basis into seven main groups such as Kaolinite, Montmorillonite, Illite etc., Mehmel (1935) Edelman and Favejee (1940) Stout (1939) and Hendricks (1938) have suggested the structures of clay minerals, particularly of halloysite, which had been said to be consisting of Kaolinite layers separated from each other by single molecular layers of water Considerable uncertainty regarding the details of the structure of Montmorillonite minerals was expressed by Hofmann Endell and Wilm (1933) According to the concept of Maegdefrau and Hofmann (1937) Marshall (1935) and Hendricks (1942) Montmorillonite has been suggested as being composed of units made up of two silica tetrahederal sheets with a central octahedral sheet of alumina.

Manguin (1928) Jackson and West (1930-33) Winchell (1925) Hendricks and Jefferson (1939) were of the view that the structure of Illite minerals consists of the same unit as that for Montmorillonite except that some of the silicons are always replaced by aluminiums and the resultant charge deficiency is balanced by potassium ions. Grim (1937) observed that the Illite clay minerals differ from the well crystallised micas, in being less substituted by $Al.^{+++}$ ions for $Si.^{++++}$

Recently Brindley and Robinson (1948-51) have considerably amplified the knowledge of the structure of chlorites and the variations among the different members of the family

According to Hendricks and Jefferson (1938) Barshad (1949-50) and Gruner (1934-39), the structure of vermiculit mineral consists of an alternation of mica and double water layers and there is always a charge deficiency due to the substitution of Al^{+++} for Si^{++++} This charge deficiency is made up by cations (Magnesium and Calcium) which occur chiefly between the mica layers and are largely exchangeable.

The structure and composition of the Sepiolite palygorskite-attapulgite minerals have been comparatively least developed Fersman (1913) Longchambon (1937) Migeon (1936) and Caillere (1936-51) have shown by intensive study that these minerals have a considerable range in the relative amounts of Ca and Mg but they give the same general X-ray diffraction and dehydration characteristics.

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In view of the above references given in brief, regarding the [illegible] complexities that are inherent in the formation of clay minerals, the structure of the most abundant types—Kaolinite Montmorillonite and Illites, have been studied in greater detail than other groups which are much more complicated.

The composition and structural characteristics of these minerals have been studied by chemical analysis and X ray diffraction patterns. Their physical properties such as cation exchange, pH value, hydration, heat of metting optical and thermal properties have also been considered to be important in throwing light on the structure and composition of clays.

In this paper we communicate our results on the complete chemical analysis of four ceramic clays known as (1) Rajmahal (2) Kasimbazar (3) Katni and Chitrakoot which were obtained from the Govt. Pottery Works, Khurja. The base exchange properties were also studied and the nature of the above clays has been discussed in the light of their chemical composition and their base exchange properties.

Experimental

Estimation of Silica, Fe, Al, Ti Ca and Mg—

The above estimations were done by following the standard method of silicate analysis as given in Scott (1952) A weighed quantity of the clay sample was taken in duplicate and fused with sodium carbonate The melt was treated with dilute HCl boiled and filtered. The insoluble silica was treated with HF and heated to remove silica as volatile SiF_4 The loss in weight corresponds to the amount of silica. This ignited mass was again fused with sodium carbonate, treated with HCl and filtered. The insoluble content was rutile and the latter filtrate was mixed up with the former for estimating Fe, Al, Ca and Mg Ti was estimated from amongst the third group precipitates by precipitating as Cupferron compound ($Ti(C_6H_5O_2N_2)_4$) loc. cit. and then igniting it to TiO_2

For the determination of the alkalies a separate portion of the clay was fused according to the method of Smith (1871) The total quantity of sod um a d potassium was determined as perchlorate Treadwell and Hall (1913) The mixture was separated by washing with alcohol and the difference in the weight gave the actual quantities of alkalies present.

Estimation ot the Anions.—

Chloride was estimated as AgCl from the sodium carbonate extract Vogel (1951) Sulphate was estimated as $BaSO_4$ and phosphate by reprecipitation of ammonium phosphomolydate precipitate as magnesium ammonium phosphate from the sodium carbonate extract Vogel (1951).

Exchangeable Bases

Exchangeable Ca and Mg were determined by leaching with normal sod um chloride solution Hissink (1923) and Gedroiz (1918) Exchangeable sodium and potassium were determined by leaching with ammonium acetate Piper (1950) Total exchangeable metal cations were determined by two [illegible]

ferent methods Schofield (1935) and Bray and Willhite (1929) Total cation exchange capacity was determined by leaching with ammonium acetate and then with a normal solution of K_2SO_4 and finally by estimating ammonia in the leachate as given in Piper (1950) Degree of base saturation was determined by employing the formula $\frac{100 \times S}{T}$ where T is the total cation exchange capacity and S is the total exchangeable bases. Similarly the individual exchangeable base saturation was determined by taking the ratio of the sum of the individual exchangeable bases (Ca Mg Na and K) and the total cation exchange capacity (T) and multiplying it by 100

pH of the clays was determined potentiometrically by using antimony electrode against calomel Vogel (1951)

TABLE 1

Percentage analysis of clays and their molecular formulae

	Rajmahal clay	Kasimbazar clay	Katni clay		Chitrakoot clay
SiO_2	27 16	25 34	29 59		23 96
Fe_2O_3	3 50	6 63	5 224		4 604
Al_2O_3	46 28	58 42	55 890		62 750
TiO_2			3 749	7 522	2 580
Rutile			3 773		
CaO	3.75	1 82	1 305		0 412
MgO	1 54	0 25	0 187		0 107
Na_2O	0 21	1 954	2 167		1 497
K_2O	1 03	1 754	1 796		2 433
CO	1 70	0 28	1 150		1 340
Cl	0 568	0 23			
SO''_4		1 067	1 064		
PO_4					0 274
C	0 077	0 029	0 087		0 109
Total	85 815	97 874	105 982		100 066
Probable Molecular Composition.	20.3CaO(SiO_2) 11.5MgO(SiO_2) Na_2O 3.3K_2O 6.4Fe_2O (SiO_2) 28.4Al_2O (SiO_2)$_2$ 107 6Al_2O	303CaO(SiO_2) MgO(SiO_2),3 1 Na_2O 3K_2O 6 8 Fe_2O_3(SiO_2)$_2$ 14.5Al_2O (SiO_2)$_2$ 80.6 Al_2O	5CaO(SiO_2),MgO(SiO_2) 7.39Na_2O 4 13K_2O 6.95 Fe_2O_3(SiO_2)$_2$ 20.21 TiO_2 26.74 Al_2O (SiO_2)$_2$ 92.36 Al_2O		2.8CaO(SiO_2)$_2$ MgO(SiO_2) 9.2 Na_2O 9 6K_2O 10.6Fe_2O_3 12.3 TiO_2 39.2Al_2O (SiO_2) 197 3 Al_2O_3

TABLE 2

Exchangeable bases and percentage base saturation

		Raj-mahal clay	Kasim-bazar clay	Katni clay	[illegible]
SiO_2/R_2O_3		0 95	0 67	0 85	0 [illegible]
Exchangeable Ca		3 40	16 81	4 269	10 15
Mg		2 18	11 82	0 0	0 0
Na		0 04	0 012	0 295	0 14
K		0 88	1 907	0 114	0 15
Sum of the individual exchangeable bases	S.I B	6 50	32 519	4 681	10 4[illegible]
Total exchangeable metal cations	Schofield	3 71	11 80	21 25	14 5[illegible]
	Bray & Willhite	2 72	9 15	13 22	16 68
Total cation exchange capacity T		15 5	48 385	15.515	47.5[illegible]
Degree of or individual exchangeable base saturation.	$\frac{SI\,B \times 100}{T}$	42 00	67 26	30 17	21 4[illegible]
Degree of, or / base saturation	$\frac{Sch \times 100}{T}$	23 94	24 38	136 96	21 [illegible]
$S \times 100/T$	$\frac{SB\,W \times 100}{T}$	17 58	18 9	85 21	33 [illegible]

TABLE 3

Ratio of exchangeable bases/Total bases

	Rajmahal lay		Kasimbazar clay		Katni clay		[illegible] clay	
Ca	0.00127		0 0144		0.00159		0 03100	
Mg	0.00731		0.0788		0.00		0 00	
Na	0 00025	.00128	0 00008	00139	0.00018	.000 56	0 0001 4	[illegible]
K	0.00103		0.00131		0.000076		0 000[illegible]3	
Total	0 00189		0.09159		0.004816		0 031[illegible]	

OBSERVATIONS

The most striking observations to be obtained from the foregoing tables are as follows —

Silica varies from 24-29 6 per cent and Fe_2O_3 varies from 3 5-6 6 per cent in the different clays Al O_3 varies more widely in the different clays except Kasimbazar and Katni, where the difference is about 3 per cent. The difference in the percentages of CaO MgO Na_2O and K_2O in the composition of Kasimbazar and Katni clays are much less than those of Rajmahal and Chitrakoot. It is further interesting to observe that Katni and Chitrakoot clays contain appreciable quantities of TiO_2 and Rutile in their composition and also that the clays contain the anions chloride (Rajmahal) Chloride and Sulphate (Kasimbazar) Sulphate (Katni) and Phosphate (Chitrakoot) Carbonate was also found to be present from 3-4 per cent. The percentage of carbon also varies from 0 03-0 11 in these clays. The ratio of $SiO_2/Al_2O + Fe_2O_3$ varied between 0 62 (Chitrakoot) to 0 95 (Rajmahal) vide table no 2

Sum of the individual exchangeable bases varied from 4 7 32 5 in the following order —

Kasimbazar > Chitrakoot > Rajmahal > Katni

Exchangeable calcium follows the same order except for Katni clay in which it is a little higher than Rajmahal. No exchangeable magnesium is shown by Katni and Chitrakoot clays. Exchangeable sodium and potassium do not vary in the same order as that of the sum of the individual exchangeable bases.

Total exchangeable metal cations determined by Bray and Willhite (loc. cit.) as well as by Schofield's (loc cit.) method, do not follow the same order as shown by the sum of the individual exchangeable bases (Ca, Mg Na and K) Total cation exchange capacities are the highest and nearly equal in the Kasimbazar and Chitrakoot clays (48 4 and 47 5) while in the Rajmahal and Katni clays these values are identical (15 5) The values of pH do not agree with the order of the degree of base saturation in these clays. The degree of base saturation from the values of total exchangeable metal cations obtained by Schofield and Bray and Willhite method falls far short of the pH values of the clays actually observed, while the degree of base saturation calculated from the values of the sum of the individual exchangeable bases has by far been found to be more significant in explaining the order of the variations of pH observed in these clays.

DISCUSSION

Since all the clay minerals are derived from the weathering of primary silicate minerals such as Orthoclase ($KAlSi_3O_8$) Hornblende ($MgFeSiO_4$) Olivine and Muscovite ($H_2KAl_3Si_3O_{12}$) the occurrence of three main types

of clay minerals viz., Montmorillonite, Illite or Kaolinite is supposed to depend on the degree of weathering and the chemical nature of the weathering complex. It has also been suggested that silicon is removed more easily by leaching than iron or aluminium except under strongly acid condition. It has further been observed by previous workers that old soils of the tropics generally have a high percentage of Aluminium, Iron and the clay fraction contains larger amount of Kaolinite. Clays of warmer climates have been found to have suffered greater losses of silicon than those of cooler climates. Our observations on the percentage of silica suggest accordingly that the Rajmahal, Kasimbazar Katni and Chitrakoot clays have suffered similar changes of weathering and are probably derived from similar mixtures of primary silicates. The theoretical composition of Kaolinite clays contains SiO_2 46.54 p.c., Al_2O_3 39.5 p.c. and H_2O 13 96 p c. that of Montmorillonite contains silica 66.7 p.c., Al_2O_3 28 3 p c. and H_2O 5 p c while Illite occurs in different forms of variable composition in which potassium, iron and magnesium have been found in relative abundance. Grim (1953) but in the samples of Rajmahal, Kasimbazar Katni and Chitrakoot clays the percentage of silica is much lower while that of Al_2O_3 goes up higher in all these samples. This observation is in conformity with the above view that the effect of temperature with the tropical soils speeds up chemical and biological reactions in the tropical regions with consequent loss of silica by leaching and gain of alumina in the composition of the clays Some fraction of these elements is undoubtedly held by the clay particles as exchangeable cations due to the residual or unsatisfied negative charges arising from the broken bonds around the silica alumina units which could be balanced only by the adsorbed cations It may also be possible that substitutions within the lattice structure of trivalent aluminium by ions of lower valency and smaller radius such as magnesium, or the hydrogen of the exposed hydroxyls may also be responsible for holding some fraction of the bases in an exchangeable form. Iron can possibly substitute Al by isomorphous replacement without affecting the net charge of the clay complex.

It is interesting that Katni and Chitrakoot clays contain TiO_2 etc. Brindley and Robberts (1946-48) suggested that Ti or Fe may substitute Aluminium in the poorly crystallised Kaolinite in which the Al positions are more mobile for substitution by these elements It appears that Ti might have entered the structure of Katni and Chitrakoot clays by such a process.

It follows from a consideration of the various factors influencing cation exchange capacity that there is no single capacity value that is characteristic of a given group of clay minerals hence it has been observed by previous workers that every group of clay minerals has a range of cation exchange capacities (m. e per 100 gms of the mineral) e.g. Kaolinite has a range of 3 15, Halloysite. 2H O 5 10 Halloysite 4H_2O 40-50 Montmorillonite 80-150 Illite and Chlorite 10-40 and Sepiolite-attapulgite-polygorskite 20-30. In the four different ceramic clays under investigation we have observed that the total exchangeable metal cations of Rajmahal are 3 71 and 7 m. e. for

Kasimbazar 11 8 and 4 15 m. e., for Katni clay 21.25 and 13.2 m e., and for Chitrakoot clay 14.5 and 15 1 m.e. by Schofi ld and Bray and Willhite s methods respectively These values obviously suggest that there is a wide discrepancy in some cases between the two methods recommended for deter mining the total exchangeable bases Assuming on reasonable grounds that the Bray and Willhite s method is more reliable, it may be concluded that the total exchangeable metal cations of these clays vary between the range of 2.72 16.1 which approximately falls within the range of 3-15 m. e per 100 gms. for the Kaolinite group already referred in the previous paragraph. Since it has been observed that types of clays are also interchangeable as a result of weathering and other physico chemical conditions, it may be concluded that the four types of clays do not belong to a particular group of clay mineral such as Kaolinite, Montomorillon te or Illite from the base exchange considerations The considerable proportions of calcium, magnesium, sodium and potassium entering into their structures lead to a more probable conclusion that these ceramic clays are compounded of the different types of clay minerals. The base exchange properties merely suggest that the Kaolinite group may be predominent and in larger proportion in the four types of clays which we have investigated. This conclusion is further supported by the fact that Katni and Chitrakoot clays hold titanium and rutile in thei composition, probably due to poorly crystalline Kaolinite group present in them. Rajmahal clay appears to have been derived from a well built Kaolinite structure having less unsatisfied charge and hence showing the lowest value of exchangeable bases while in others the exchangeability becomes higher or lower in proportion to the different types of clay crystals having different amounts of unsatisfied charge

It is interesting to refer to the sum of the individual exchangeable bases of Rajmahal 6 5 m. e. and Kasimbazar 32.5 m. e. which are much higher than the values for the total exchangeable metal cations determined by the Schofield and Bray and Willhite's method, while those of Katni and Chitrakoot clays are less than the total exchangeable metal cations determined by the same methods. These abnormalities in the relation of the sum of the individual exchangeable bases and the total exchangeable metal cations are not uncommon which we have previously communicated (1954)

It may be argued that the base exchange capacities of the clays depend upon the percentage of base saturation. Our observations on the degree of base saturation do not support this fact strictly hich means that there are many other causes to influence the base exchange phenomenon. Marshall (1949) has observed that the percentage of ionisation of adsorbed cations depends upon the particular clay mineral the concentration of clay water system, the nature and relat e concentration of the cations and lastly on the nature of adsorbed anions also In all the fou ceramic clays under investigation we conclude a priori that th exchangeable bases in these lays may be influenced by the Cl^- SO_4^{--} or PO_4^{---} and also by CO_3^{--} ions as the case

may be. These negative ions in the clay surface stand in the way of the sorption of some metallic bases by surface reaction and formation of soluble salts. It can however be anticipated that hydrogen would be attracted more readily because of its greater affinity for the anions and small ionic radius. Such a possibility is supported by the values of the total cation exchange capacity T given in table no 2 where Kasimbazar clay containing SO_4 and Cl and Chitrakoot clay containing PO_4 ions have the highest values, because of considerable increase in the exchangeable hydrogen in these samples.

The degree of base saturation calculated by Schofield and Bray and Willhite s method hardly bears any correspondence to the base exchangeabilities In the case of Katni clay the degree of base saturation is 156% which apparently seems to be absurd showing that Schofield's method may lead to very faulty conclusion On the contrary the degree of base saturation calculated from the sum of the individual exchangeable bases bears a clear relationship with the total individual exchangeable bases in Rajmahal, Kasimbazar and Katni clays. Discrepancy however arises in the case of Chitrakoot clays, which is due to the excess of exchangeable calcium alone. The presence of the PO_4 ions in the Chitrakoot clay may be responsible for the formation of calcium phosphate which may be leached out by sodium chloride in the determination of exchangeable calcium. We are therefore, of the opinion that in the absence of anionic reactions the degree of base saturation calculated from the ratio of the sum of the individual exchangeable bases is, by far more reliable to explain the exchangeability than the methods of Schofield and Bray and Willhite which have so far been advocated. The values of base saturation obtained by estimating the individual exchangeable bases should, therefore, prove to be a more useful characteristic for the soils and clays in respect of their base exchange properties.

Our observations given in table 3 showing the relation between pH and the exchangeable bases lead to the interesting inference that exchangeable Na and K are more directly connected with the pH values of Rajmahal, Kasimbazar Katni and Chitrakoot clays. The table shows the exchangeable fraction of each base in the clay which was calculated by the ratio of the exchangeable base in milli equivalents/total percentage of the base in the clay. It is observed that the order of increase of pH was in accordance with the values of the exchangeable fractions of (Na+K) calculated as mentioned above The exchangeable fractions of (Na+K) were roughly 0.0131, 0.11, 0.0076 0.0024 for Rajmahal, Kasimbazar Katni and Chitrakoot clays respectively and surprisingly enough their corresponding pH for these clays were 9.25 9.4 8 7.7 No such correspondence however is observed with the exchangeable fraction of Ca or Mg Although the percentage of base saturation has been observed to be directly connected with the pH values of clays and soils the data of our investigations are fairly convincing that the exchangeable fraction of (Na+K) in the clay may be more directly connected with the variations of pH than the exchangeable bases Ca and Mg

On comparing exchangeable fractions for (Na+K) of Kasumbazar Katni and Chitrakoot clays we find that there is a regular decrease in pH with the decreasing exchangeable fraction This, however may not be the only factor to influence the pH values yet the co-relation observed is interesting enough

The foregoing observations on the percentage composition and the exchangeable bases give fairly convincing data for the characterisation of the ceramic clays. There are striking points to suggest in the light of our discussion that the clays under investigation belong to a mixed group of clay minerals.

References

1. Barshad, I (1948) Vermiculite and its relation to Biotite, *Am. Mineral* 33 655-678.
2. Barshad I (1949) The Nature of Lattice Expansion and Its Relation to Hydration in Montmorillonite and Vermiculite, *Am. Mineral* 34: 675-684.
3. Barshad, I. (1950) The Effect of Interlayer Cations on the Expansion of the Mica Type Crystal Lattice *Am. Mineral.*, 35 225-238.
4. Bray R. H. & Whillhite, F M (1929): *Ind. Eng Chem. (Anal Ed.)* 1: 144
5. Brindley G W (1951) The Kaolin Minerals, "X Ray Identification and Structure of Clay Minerals, Chap 2 pp. 32 75, Mineralogical Society of Great Brital Monograph.
6. Brindley G W & K. R Robinson (1946) Randomness in the structure of Kaolinit Clay Minerals, *Trans Faraday Soc* 42 B 198-205
7. Caillere S. (1936) Etude de quelques silicates magnésiens à facies asbestiforme ou papyrace n'appartenant pas d groupe de l'antigorite, *Bull. Soc Franc, minéral.*, 59 353-386.
8. Caillere, S. & S. Henin (1951) Palygorskite-Attapulgite "X ray Identification and structure of clay minerals Chap 9 234-243, Minerological Society of Great Britain Monograph.
9. Edelman C. H. & J C. L. F vejer (1940) On the Crystal Structure of Montmorillonite and Halloysite Z. Krist, 102 417-431
10. Fersman, A. (1913) Recherches sur les silicates de magnésie, *Mem. Russian Acad. Sci.* 32 377 392.
11. Gedroiz, K K. (1918) Zhur Opit. Agron 19 226-244
12. Grim, R. E. (1953) Clay Mineralogy p. 129
13. Grim, R. E. R H. Bray & W F Bradley (1937) The Mica in Argillaceous Sediments, *Am. Mineral*, 22: 813-829
14. Gruner J W (1934): Vermiculite and Hyrobiotite Structures. *Am. Mineral* 19 557 575.
15. Gruner J W (1939) *Am. Mineral*, 24 42.-433.
16. Hendricks, S. B. & M. E. Jefferson (1938) Structure of Kaolin and T c, Pyrophyllite Hydrate and their Bearing on W ter Sorption of the clays. *Am Mineral*, 23 863-875
17. Hendricks, S B. (1939) Polymorphism of the Micas with Optical Measurements. *Am. Mineral.*, 24 729-771
18. Hendricks, S. B. (1938) Crystal structure of Vermiculite and Mixed Vermiculite Chlorites. *Am. Mineral* 23 851-863
19. Hendricks S. B. (1942) Lattice Structure of Clay Minerals and some properties of clays, *J Geol* 50 276-290
20. Hissink, D J (1933) *Soil. Sci* 15 269-276.

21 Hofmann U., K. Endell & D. Wilm (1933): Kristallstruktur und Quellung von Montmorillonit. Z. Krist., 86: 340-348

22. Jackson, W W & J West (1930): The Crystal Structure of Muscovite. Z. Krist., 76: 211 227

23. Jackson, W W (1933): The Crystal Structure of Muscovite. Z. Krist., 85: 160-164

24 Journal of the Indian Society of Soil Science (1954) 2 December pp. 131-133.

25 Long Chambon, H. (1937): Sur certaines Caractéristiques de la Sepiolite d' Ampandrandava et la formule des sepiolites. Bull. Soc. Franc. Mineral., 60: 232-276

26. Marshall, C. E. (1935): Layer Lattices and Base Exchange Clays. Z. Krist., 91: 433-449

27 Marshall C. E. (1949): The Colloid chemistry of Silicate Minerals.

28. Maegdefrau E. and U Hofmann (1937): Kristallstructur des Montmorillonits, Z. K. 98, 299-323.

29 Mauguin, C H. (1927): Etude des micas au moyens des rayons X. Compt. rend., 185: 288-291

30. Mauguin, C H (1928) Etude des micas au moyens des rayons X. Bull. Soc. Franc. Mineral, 51: 285-332.

31 Mehmel, M. (1935): Ueber die struktur von Halloysit und Metahalloysit Z. Krist., 90: 35–43.

32. Migeon, G (1936): Contribution á l' é tude de de la définition des Sepiolites. Bull. Soc. Franc. Mineral. 59: 6-133.

33. Pauling, L. (1930): The structure of Micas and Relative Minerals Proc. Nat. Acd. U S., 16: 123-129.

34. Piper C. S (1950): Soil and Plant analysis 171 176-181 and 187-189.

35 Robinson, K. & G W Brindley (1948): Structure of Chlorites. Proc. Leeds Phil Lit. Soc. Sci., Sect 5: 102 108.

36. Robinson K. & G W Brindley (1951): X-ray Identification and structure of the clay minerals. Chap. 6 pp. 172 198 Mineralogical Society of Great Britain Monograph.

37 Schofield, R. K (1933): J Agric Sci 23: 252-254

38. Scott, W W (1952): Standard methods of chemical analysis. 1: 814-815. 959-960 and 532 533

39 Smith J Lawrence (1871): Am. Jour Sci 2: 50 269 and Am. Chem. Phar. 159-62.

40. Stout, P P (1939): Alterations in the Crystal structure of the clay minerals as a result of phosphate fixation soil Sc. Soc Am. Proc 4: 177 182

41 Treadwell E. P & Hall, William T (1915): Quantitative analysis, pp. 61-63.

42. Vogel Arthur I (1951): Quantitative analysis, pp. 399-400, 497-499 and 676-681

43 Winchell, A.N (1925) Studies in the Mica Group Am. J Sci., ser 5, 9: 309-327, 415–430.

ON THE BIOLOGY AND IMMATURE STAGES OF *CADMILOS RETIARIUS* DIST., A SAP SUCKER ON COMPOSITAE (HETEROPTERA : TINGIDAE)*

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CONTENTS

INTRODUCTION

Out of the 287 species belonging to 67 genera of Tingids so far recorded in the Oriental region (Drake 1960) our knowledge on the biology and immature stages has been limited to hardly of six species. No clear record of the periodicity and the host plants of the various species is available, as the collections were made mostly by using light traps. Realising the extensive damage caused by the Tingids mostly to garden plants especially during the summer months, the biological aspects of various species are attracting attention of Entomologists in the recent years in India. The main difficulty which the workers face to understand the various phases of the biology of these insects is the lack of proper mass rearing techniques. The following notes give a broad outline of certain aspects of the biology of *Cadmilos retiarius* Dist. with the description of the egg and immature stages. Most of the observations were made in the field.

HOST PLANTS AND INCIDENCE

C. retiarius first makes its appearance on *Helianthus annuus* during the middle of March when stray cases of adults are found feeding on the upper surface of the leaves. Fresh eggs are laid on these leaves during the end of March and

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the population steadily increases on this plant during the month of [illegible] During the middle of May they spread on the adjoining annuals belonging to the same family namely Compositae. The plants mainly attacked are *Gaillardia*, *Daisy* *Chrysanthemums* *Marigold*, *Vernonia* and *Lettuce*. Spreading on all these plants takes place simultaneously with the multiplication phase on *Helianthus*. As the adults are not capable of long flights it is highly probable that the natural agencies like winds aid in dispersal, including the immature stages. All these plants are heavily infested during the month of July. By the middle of August there is a steady decline in the population, disappearing completely by the beginning of September when the seasonals completely decay. The density of population depends on the onset of monsoon. It is observed that in 1958-59 when the monsoon was in time the peak of the population was reached during the end of June (soon after the first fall) but in 1959-60 due to delayed monsoon there was a fall in the population density during the beginning of July which shot up steeply soon after the monsoon (middle of July). During the peak period of population a single leaf carries an average of three adults and six immature insects. At the end of July most of the adults and nymphs feed on flower buds and on stems of succulent herbs (*Marigold*, *Lettuce* and *Vernonia*). When their natural food plants are destroyed during the peak of the population (July August) they attack wild plants especially *Argemone mexicana* (Papaveraceae). The stage at which over wintering takes place is not known.

Copulation and Egg Laying

Usually a single leaf bears a couple of copulating pairs which is observed mostly in the mornings and evenings but never in mid-day. The whole process lasts for about half an hour.

The eggs are deposited on the upper surface of the leaf, on the [illegible] of bracts and in the stems of succulent herbs. The site of egg laying and the average number of eggs on a particular site in the various host plants are summarised in table 1. The eggs are inserted slantingly into the plant tissue exposing only the operculum but in rare cases neck or collar is visible above the level of the surface when the eggs are inserted vertically. The region of the plant tissue where the eggs are inserted especially in *Helianthus* shows parched, [illegible] kish patches the exposed opercular region appearing as white or brownish [illegible]

Description of the Egg

Length 0.48mm width 0.22 mm. The egg shows three distinct regions, the operculum or the cap, the neck or the collar and the body.

The operculum (Fig. 2 and 3) is slightly oval (0.11 mm wide) and is composed of three parts viz —the sealing bar (SB), the opercular plate (OP) and the spongy opercular crest (S). The spongy rim or the sealing bar rests on the spongy region of the chorionic rim. In normal condition at the time of hatching the operculum with the sealing bar is lifted up with a portion of the endochorion (embryonic cuticle ?) attached to it keeping connection with the empty exochorion which remains inside the plant tissue. The inner plate of

TABLE 1

Statement showing the site of egg laying and the average number of eggs on the particular site of the various host plants of Cadmilos retiarius Dist

Host plants (Compositae)	*Site of egg-laying*	*Average number of eggs*
Chrysanthemums	Under surface of leaves, submarginal area	Scattered or in groups of five eggs on a single leaf.
Daisy	Upper surface of the leaves and the involucre of bracts.	Scattered, two eggs on a single leaf
Gaillardia	Mostly on the upper surface of the leaves usually at the leaf tip and bracts.	Scattered or in groups two eggs on a single leaf.
Helianthus	Upper surface of the leaves, submarginal area and involucre of bracts.	Groups of seven eggs on leaves, or singly on bracts.
Launea	Upper surface of the leaves.	Scattered two eggs on a leaf
Mangold	No eggs or immature stages found.	
Vernonia	Stems and leaves.	Scattered, two on a single leaf.

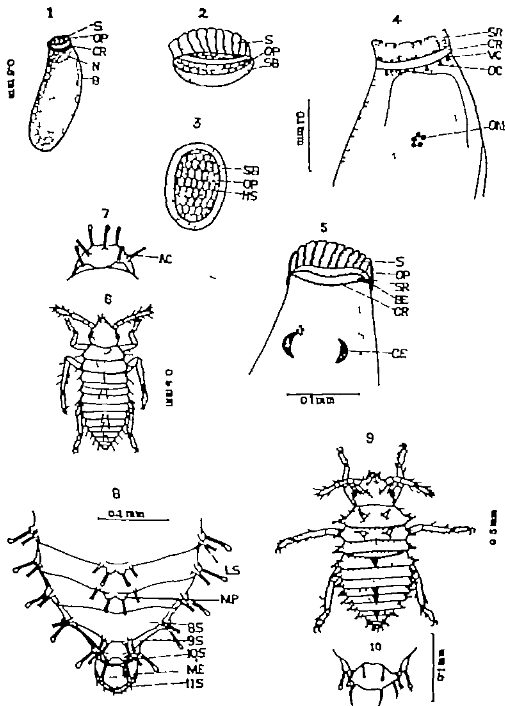
the opercular plate is very dark with hexagonal sculpturations (Fig 3 HS) the sealing bar forming the rim. The outer phase is formed by the white spongy crest (0 035 mm. high) which represents the exposed region of the egg

Crosby and Hadley (1915) reported in the egg of *Leptobyrsa explanata* Heid. a "scab" over the cap or operculum which falls off several days before the egg hatches. Dickerson and Weiss (1916) in *Leptoypha mutica* Say reported a brownish "scab" like crust deposited over the cap by the parent insect. Tilden (1950) observed in *Corythuca morilli* O & D a droplet of dark fluid having lower specific gravity than the faecal matter covering the area around the wound into which the eggs are being laid, forming a sealing medium which prevents desiccation. Roonwal (1932) considered the spongy region of the chorionic rim as the "problematic structureless tissue" lying above the opercular rim (chorionic rim) and considered the whole region above the chorionic rim as the operculum with the micropylar canals traversing around it.

According to Beament (1946) the sealing bar which fits on the spongy region of the chorionic rim is characteristic of the Cimicomorpha. Southwood (1956) proposed the name pseudoperculum to the so called operculum (without the sealing bar) of the Pentatomorpha. Roonwal's description of the egg of *Teleonemia scrupulosa* Stål is of Pentatomorphic type which is uncommon in the eggs of Tingids studied by the author Southwood (1956) is of opinion that the true operculum of Cimicomorpha with the weak point in the sealing bar aids in the eclosion of the larva and its presence is correlated with the absence of the median egg burster in this group

As there is no sign of deposition of faecal matter over the operculum the spongy 'opercular crest' should not be confused with the "scab" of the earlier writers. Further this crest forms the part of the operculum separated from the rest of the egg by the opercular plate. No canal traverses through this plate, but there are pores all over the crest which are very much widened and most in the eggs examined during the rainy season The eggs examined during the dry weather however showed the crest much shrunk, almost to the level of the opercular plate. The outlines of the vertical canals which always run along the rim of the egg are very faintly marked in the sealing bar and the spongy region of the chorionic rim making the point clear that there is no direct connection between the pores of the crest and the vertical canals. The obvious function which we could deduct from the structure of the opercular crest is to protect the minute respiratory openings in the mesh work of the sealing bar from getting during the rainy season As the pores of the crest are wide, easy drying could be possible during this season But desiccation is prevented by the spongy region of the chorionic rim that lies in the plant tissue, which in all host plants of this species is succulent and also by the shrinking and hardening of the crest Thus the main functions of the opercular crest appear to be moisture regulation or control of humidity and prevention of desiccation Then the question arises whether the eggs of other Tingids are also provided with similar mechanism? Out of the ten species of Tingids eggs examined by the author in nine cases the site of deposition of the egg (mainly leaf) is not succulent as that of *Cadmilos retiarius* In most cases the eggs are laid under the surface of the leaf and in a few cases, especially *Dictyla sufflata* the eggs are laid on the upper surface. In the latter case the scab (deposition of faecal matter over the operculum) is found. Further the backward extension of the crest (Fig 5, BE) surrounding the chorionic rim is very significant only in *Cadmilos retiarius* which is very suggestive of the above mentioned functions

The *neck* or the *collar* (Fig 1 N) is the portion of the chorionic rim which is fringed by the spongy *region* (Fig 4 SR) (0 035 mm high) The spongy region shows faint outlines of about twelve vertical canals which open independently just below the chorionic rim (Fig 4 VC) The neck region just below the chorionic rim which faces the surface is slightly indented and darkly pigmented and is more tough in texture than the rest of the chorion.



1. Side view of the egg. 2. Side view of the operculum. 3. Inner view of the operculum. 4. Anterior region of the egg without the operculum. 5. Anterior region of a parasitised egg. 6. First nymphal instar. 7. An-

teclypeal region of the first nymphal instar 8 Posterior abdominal region of the first nymphal instar 9 Second instar nymph. 10. Ninth and tenth abdominal segments of the second instar nymph.

B. body BE. backward extension of the opercular crest CE. crescent shaped eye of the parasite CR. chorionic rim HS hexagonal sculpturation LS elongated slender scolus with cupped tip ME. membrane MP median short tuberculated globulated spines of the sixth abdominal segment N. neck, OC. opening of vertical canal OM. Ommatidia OP opercular plate S. opercular crest SB sealing bar SR. spongy region of the chorionic rim VC. vertical canal 8s 9s 10s 11s. last four abdominal segments.

The body (Fig 1 B) of the egg is not very smooth but bluntly rounded at the posterior end. Hexagonal sculpturations are marked but not uniformly. The outlines of the sculpturations are more pronounced along the portion of the egg facing the surface and also around the region just below the collar. Not all eggs bear the pigmented outlines

Hatching

Most of the eggs belonging to the same group hatch almost at the same time. At the time of hatching, due to the enlargement of the egg the operculum separates at the point of attachment of the sealing bar with the spongy region of the chorionic rim Within fifteen minutes there is a sudden outpushing of a conical transparent projection visible above the level of the chorionic rim. Pulpitation goes on rhythmically at this region and within five minutes the labrum spreads out, followed by the basal segments of the rostrum with its distal segment still within the chorion Below the level of the labrum the five red ommatidia appear The wriggling movements of the freed head bring the whole body out within fifteen minutes, the distal ends of the legs still retaining the connection with the chorion. Then the first pair of legs are freed. After getting firm grip on the surface of the leaf with the freed legs the other legs are pulled out. The nymph after walking about for a few minutes, makes efforts to feed, followed by the erection of the spines. A more or less similar type of hatching is described by Johnson (1936) in the case of *Leptobyrsa rhododendri* Horwath.

Immature Stages

First instar—(Figs 6-8) Length 0.68 mm. Width 0.27 mm.

Head—Length 0 17 mm. Width across the eyes 0 19 mm. Anteclypeus visible from above as conical projection with three or four long slender globulated spines fringing the margin (Fig 7) and a couple of long slender scoli. Two short tuberculated, globulated spines (0 038 mm long) on either side of the base of the anteclypeus similar spines, one on the middle of the postclypeal region and one on either side of the vertex near the lower level of the eye The junction of the arms of the Y-shaped streak marks the posterior extremity of

the postclypeus. Antenna 0.22 mm long, four segmented the scape and pedicel of equal length and the two annuli of the flagellum of equal length (0.06 mm) the terminal annulus with sharp tip surrounded by short nonglobulated spines and its length with six to eight elongated slender nonglobulated spines (0.04 mm) the first annulus also with four to six similar spines. Eyes with five red ommatidia with a couple of short, globulated spines in between them (Fig 16) Rostrum, four segmented, extending upto the second abdominal segment.

Thorax.—Prothorax, a little less than half the size of the entire thorax all three segments with a short tuberculated, slender globulated spine on the outer margin pro and meso-thorax with a similar spine on either side of the mid dorsal streak prothoracic outer margin with an additional slender long scolus the tip of which is cupped (Fig 8, LS)

Abdomen.—(Fig 8) Ten segments visible the first segment shows fusion with the second segment exposing only the mid tergum the lateral margins of the segments two to nine with a short tuberculated, slender globulated spine, at the base of which arises a slender long scoli a couple of slender elongated globulated spines fringing the posterior margins of segments nine and ten eleventh segment telescoped within the tenth segment, the dorsal and ventral sclerites, fringed with short spines appear as flaps attached to the anus during defaecation The segments two, five, six and eight with a pair of short tuberculated, slender globulated spines on the mid-dorsal line, at the base of each tubercle arises an elongated slender scoli. Segments four and five with the openings of the dorsal scent glands anteriorly

Second Instar.—(Figs 9-10) Length 0·79 mm. Width 0.34 mm.

Head.—Length 0 17 mm. Width 0·24 mm. The tubercle on either side of the anteclypeus elongated to the level of the latter the tubercles appear stout and the globulated spines at their tips become shorter (0·022 mm long) the anterior margins of the head with very short, sharp scoli and two or three elongated slender scoli. Antenna 0.31 mm. long, the distal annulus slightly longer than the proximal one. Eyes with eight to ten ommatidia bearing a couple of spines. Rostrum extending up to the first abdominal segment, the terminal segment brownish.

Thorax.—Prothorax half the size of the entire thorax the metathorax the smallest the middorsal short tubercles of the pro and meso-thorax much elongated and brownish, with short, sharp scoli along their lengths giving a serrated appearance short, sharp scoli and elongated slender scoli added at the base of each tubercle and at the outer margin of the prothorax. Legs of equal size the femur and tibia of equal length cla vs well formed.

Abdomen.—The posterio margin of each segment fringed with short sharp scoli the lateral marginal tubercles elongated and serrated, bearing very short globulated spine apically the lateral marginal tubercles of the ninth segment point upwards and placed submarginally The dorsal pair of globulated spines

Thorax.—Prothoracic anterior extension forms a hood or vesicle covering the whole of vertex, the median carination following the vesicle extending up to the tip of the proscutellum as in the adult the paranotal fold well marked dark pigmentation on either side of the median carinations and at the anterior outer margin Wing pads extend up to the fourth abdominal segment. The prothorax lost the lateral marginal tubercle. The upwardly directed lateral marginal tubercle of the mesothorax (wing pad) now lies at level with the posterior lateral margin of the second abdominal segment. Posterior margin of the prothorax (scutellum) extends further back so that the three thoracic segments approximate in the mid region with a concave notch at the posterior mid margin of each (Fig 13 N) The wing pads with fewer scoli than the prothorax.

Abdomen—The first abdominal segment much reduced and lies almost in line with the posterior margin of the metathorax the second segment also approximate with it the mid region of the terga of the first four segments exposed, the outer margins being covered by wing pads the segments two and three lose their lateral marginal tubercles the dorsal scent gland openings more widened (0 05 mm) all tubercles more or less of the same length but more elongated than those of the fourth instar

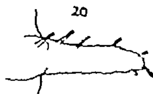
REMARKS ON THE IMMATURE STAGES

All the instars at the time of ecdysis are whitish or pale yellowish, gradually gaining brownish tinge along the lateral margins of the segments and at the base of each dorsal tubercles The first instar is olive green Congregation on the under surface of the leaf is significant. No parental care has been observed. Each instar about half an hour before ecdysis stops feeding gets firm grip on the substratum and remains with its head drooping and antennae raised Splitting of the ecdysial cuticle takes place at the junction of the arms of the Y-shaped streak following the pulsating movement at the cervical region. The whole process lasts for about twenty minutes including the withdrawal of the appendages from the cast

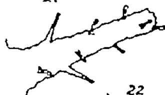
The cuticular structures are classified as follows —

1 Slender nonglobulated spines (Fig 23)
2 Short sharp scoli (Fig 19 SS)
3 Sharp scoli of medium length (0.03 mm.) (Fig 19 MS)
4 Long slender scoli with cupped tip (0.07 mm.) (Fig 19 LS)
5 Short globulated spines (0 021 mm) (Fig 17)
6 Long slender globulated spines mounted on short (Fig 18) tubercles (0.38 mm)
7 Serrated tubercles with short, globulated spines (length ranges from 0 038 mm to 0 27 mm) (Figs. 19-22)

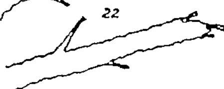








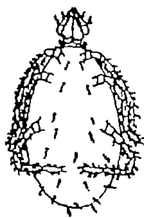


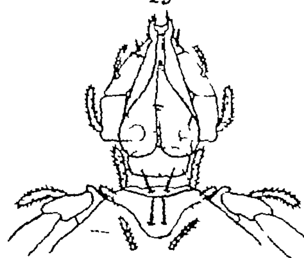




0.1mm







16, eye of the second instar nymph 17-21 median clypeal tubercle of the first to the fifth nymphal instars 22 and 23 prothoracic dorsal tubercl and the non-globulated spine of the antennal flagellum of the last instar nymph.

GS, globulated spine LS long slender scolus with cupped tip MS, scoli of medium length OM Ommatidia SS, short sharp scoli.

Fig 24 —Ventral view of *Leptus* sp an ectoparasite on *Cadmilos retiarius*.

Fig 25 —The same, cephalothoracic region, magnified.

The globulated spines of the first instar are longer than those mounted on serrated tubercles of the succeeding instars. All structures except the short scoli and slender non-globulated spines bear waxy globule at the tip.

PARASITES

The advanced eggs which were examined during the late monsoon (middle of August 1960) showed two hemispherical red patches (0.038 mm. long and 0.027 mm. broad) below the neck region, visible through the chorion (Fig 3, CE). Close examination revealed these to be of the nature of compound eyes. Southwood and Scudder (1956) recorded two eggs of *Tingis ampliata* H. S. found to be parasitised by a Mymarid (Hymenoptera) (*Anaphes* sp) and observed the formation of typical compound eye (instead of the usual five ommatidia of the first instar nymph) several days before hatching In *Cadmilos retiarius* the usual five ommatidia of each eye are also found just atop this Compound eye in return eggs of the earlier stage. It is interesting to notice that these 'Compound eyes' are formed in the horizontal axis instead of the usual formation of the paired eyes in the vertical axis of the egg This egg parasite in *C. retiarius* is now fully traced to be of the genus *Trichogramma* (Hymenoptera)

An Acarina parasite belonging to the genus *Leptus* of the family Erythraeidae is found attached both to the adults and immature stages (Figs. 24 and 25). A maximum of seven of these orange coloured parasites are found attached to the body and the appendages of a host. The parasitised last nymphal instar gives rise to adults with crinkled wings Narayanan and Khot (1959) recorded a *Garmania* sp (Mesostigmata) of mite predating on the eggs and nymphs of locust. This appears to be the first record of an Acarinid parasitising a Tingid.

KEY TO THE NYMPHAL INSTARS of *Cadmilos retiarius* DIST.

1 Wing pads not developed 2
Wing pads developed 3

2 Eyes with only five ommatidia abdominal segments two, five, six and eight, each with a pair of short tuberculated, globulated spine on the mid—dorsal line. FIRST INSTAR

Eyes with eight to ten ommatidia abdominal segments two, five, six and eight, each with a single tubercle on the mid-dorsal line SECOND INSTAR

3 Eyes with not more than fifteen ommatidia, with the couple of globulated spines in the middle of each eye wing pads never extend beyond the first abdominal segment. THIRD INSTAR

Eyes with more than twenty ommatidia without the couple of globulated spines wing pads extending beyond the first abdominal segment. 4

4 Wing pads rounded, covering the lateral margin of the first two abdominal segments metathorax and the abdominal segments with the lateral marginal tubercles. FOURTH INSTAR.
Wing pads elongated, covering the lateral margins of the first four abdominal segments metathorax and the second and third abdominal segments without the lateral marginal tubercles FIFTH INSTAR.

ACKNOWLEDGMENTS

The author is very thankful to Dr T Singh, Professor of Zoology and Entomology School of Entomology St. John's College, Agra for constant encouragement and guidance, Dr Santokh Singh for kindly going through the manuscript and Principal Shri P T Chandi for permission to work in the School of Entomology Grateful thanks are also due to the Ministry of Scientific Research and Cultural Affairs, Government of India for award of Scholarship during the tenure of this work.

SUMMARY

The incidence of the Tingid species *Cadmilos retiarius* Dist. on seven host plants has been studied. A detailed description of the egg and immature stages given with a classification of the cuticular structures and a key to the identification of the nymphal instars The spongy opercular crest cannot be mistaken for "Scab" of the former workers. Its function has been suggested to be of humidity control.

REFERENCES

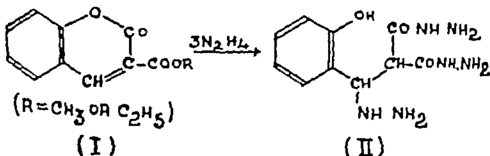
1 Beament, J W L. 1946 The formation and structure of the chorion of the egg of Heteropteran Rhodnius prolixus. Quart. J Micro, Sci., 87 393-439
2. Beament, J W L. 1947 The formation and structure of the micropylar complex in the egg shell of Rhodnius prolixus Stål (Heteroptera Reduviidae) *J Expt. Biol.*, 23 213-233.
3 Crosby C. R. & Hadley 1915. On the Rhododendron Lace bug, Leptodbyrsa explanata *Heid J Econ.Ent* Ent. 8 409-414
4 Dickerson, E L. & H. P Weiss. 1917 The Azalea Lace bug, Stephanitis pyrioides Scott (Tingitidae : Hemiptera) *Ent. News*, 28 101 105.
5 Dickerson. E. L. & H. B. Weiss. 1916. Notes on Leptoypha mutica Say (Hemip.) *Ent News* 27 308-310.
6. Distant, W L. 1910. The Fauna of British India including Ceylon & Burma, Rhynchota 5 (Heteroptera Appendix) pp. 100-126.
7 Drake. C. J & F A Ruhoff. 1960 Lace bug genera of the world. *Proc. U S. Nat Mus.* 112 (3431) 1 105.
8. Drake, C. J & N. T Davis. 1960 The morphology Phylogeny and Higher Classification of the family Tingidae, including the description of new genus and species of the subfamily Vianaidinae (Hemiptera : Heteroptera) *Entomologica Americana* 39 (n. s.) 1 100.

ACTION OF HYDRAZINE HYDRATE ON ESTERS OF COUMARIN 3-CARBOXYLIC ACID

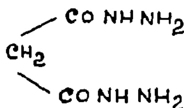
G. N. HAKSAR
S. P. KATROLIA
Jiwaji Industrial Research Laboratory Gwalior

Unsuccessful attempts were made to prepare coumarin-3-carboxyhydrazide by Baker Haksar and McOmie (J Chem Soc., 1950, 170) by the action of hydrazine hydrate on methyl or ethyl coumarin 3-carboxylate in an alcoholic solution. In either case they obtained o-hydroxy benzylidene azine (salazine) and malonic dihydrazide. Although coumarin-3-carboxyl chloride readily yielded the amide by treatment with aqueous ammonia, it did not give the hydrazide on treatment with the much stronger base hydrazine the only product isolated from the viscous reaction mass was o-hydroxy benzylidenezine. They assumed that the reaction formally involved the breakdown of the α-pyrone ring, under the influence of the strongly basic hydrazine, into the components from which it was formed, namely salicylaldehyde and a derivative of malonic acid. This reaction has been re-investigated and the course of the reaction has now been studied.

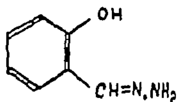
Widman (Ber 1919 52, 1658) has observed the formation of salazine from coumarin derivatives under identical conditions. Darapsky *et al.* (J pr Chem., 1936, 147 148) have found that coumarin undergoes ring fission in an alcoholic solution with hydrazine hydrate and one more molecule of hydrazine adds on to the β-carbon atom across the double bond as in the case of cinnamic acid hydrazide (Muckermann J pr Chem., 1911 83 513) Thus the action of hydrazine hydrate on coumarin 3-carboxylic esters (I) would be initially expected to give (II)



We have not been able to isolate (II) but under milder conditions of reaction, malonic dihydrazide (III) and salicylaldehyde hydrazone (IV) have been isolated.

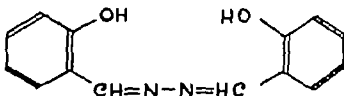


(III)



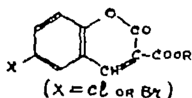
(IV)

The hydrazone (IV) is readily converted into o-hydroxybenzylidene-azine or salazine (V)

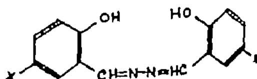


(V)

The reaction was further extended to the esters of 6-chloro-or 6-bromo-coumarin 3-carboxylic acid (VI) in order to study the effect of the substituent



(VI)



(VII)

halogen atoms on the stability of the α-pyrone ring in the coumarin nucleus towards the strongly basic hydrazine. As anticipated the inductive effect due to the halogen atom being greater than the stabilising electromeric effect due to the lone pair of electrons on the halogen atom fission of the α-pyrone ring took place. As a consequence, malonic dihydrazide (III) and the dihalogenated salazine (VII) were produced which were isolated and identified. The dihalogenated salazine was obtained when 6-chloro or 6-bromo-coumarin-3-carboxyl chloride was used in place of the ester

EXPERIMENTAL

Esters of coumarin-3-carboxylic acid

Coumarin-3-carboxylic acid was prepared by the Knoevenagel condensation (Ber., 1898, 31, 2618) from salicylaldehyde and malonic acid.

The acid was esterified with absolute methanol or ethanol in presence of hydrogen chloride by the Fischer-Speier method (Baker et al., loc cit) when methyl or ethyl coumarin 3-carboxylate was obtained, m.p. 116°C and 94 C respectively

Coumarin-3-carboxyl chloride (Boehm, Arch. Pharm., 1933, 271 490)

It was prepared from coumarin 3-carboxylic acid and thionyl chloride. Crystallisation from dry benzene gave pale shining prismatic needles, m.p. 147°C.

Action of hydrazine hydrate on esters of coumarin 3-carboxylic acid

No reaction took place when an alcoholic solution of methyl or ethyl coumarin-3-carboxylate was treated with 60 % hydrazine hydrate in the cold, even on standing for 24 hours or longer the esters were recovered unchanged on dilution with water

Methyl coumarin-3-carboxylate (2gm) was dissolved in methanol (10 ml) by gentle warming and after cooling the solution, 60% hydrazine hydrate (2 ml) was added. After refluxing on a hot water bath (boiling) for 30 minutes, glistening yellow leaflets separated It was allowed to stand and cool. Separation of the yellow solid was followed by that of a white solid. After filtering, the mixture was treated with hot ethyl acetate in which the white solid did not dissolve. The yellow solid readily crystallised from hot ethyl acetate giving lemon yellow fibrous needles, m. p ?15 C. An authentic specimen of salazine (Borsche, Ber., 1921 54, 668) did not give any depression in a mixed melting point determination. The white product was washed with warm ether to remove any unchanged ester and then crystallised from ethanol giving hite needles, m. p 152°C. Mixed melting point with an authentic specimen of malonic dihydrazide (Curtius et al., J pr Chem., 1894 51 187) did not show any depression.

Similar result was obtained when ethyl coumarin 3-carboxylate was used or when varying strengths of hydrazine hydrate were employed.

Coumarin-3-carboxyl chloride under identical conditions, gave salazine and a sticky yellow mass which could not be crystallised from the usual organic solvents.

Isolation of the hydrazone of salicylaldehyde (IV)

Methyl or ethyl coumarin 3-carboxylate (2 gm) was dissolved in warm methanol (10 ml) and then 60% hydrazine hydrate (2 ml) was added. The mixture was heated carefully on a water bath under reflux at 50°-60°C for only

5 minutes. It was rapidly cooled in ice-cold water when a pale coloured solid separated. Crystallisation from ethanol gave a solid, m. p. 90°C (Found C =61.96 / H=4.92% N=20.82% $C_7H_7ON_2$ requires C=62.22 H=5.18 N=20.74%) Mixed melting point with an authentic specimen of the hydrazone of salicylaldehyde (Cajar, Ber., 1898, 31 2806) did not show any depression. On heating above its melting point, it was rapidly converted into the azine.

Methyl 6-chloro-coumarin-3-carboxylate

5-Chlorosalicylaldehyde (Biltz and Stepf, Ber. 1904 37 4024) was condensed with malonic acid (Knoevenagel *loc cit.*) to obtain 6-chloro-coumarin-3-carboxylic acid.

The acid (2gm) was added to absolute methanol (50ml) and hydrogen chloride was passed through the suspension for 15 minutes when the acid went into solution. It was refluxed on a hot water bath for 4-5 hours and then poured into water (100 ml). A thick white solid separated. Precipitation was complete after allowing to stand for one hour and stirring from time to time. It was filtered, washed with water and crystalised from methanol. White needles, m. p. 154°C, were obtained. (Found Cl=14.56% $C_{11}H_7O_4Cl$ requires Cl =14.86%)

Methyl 6-bromo-coumarin-3-carboxylate

5-Bromosalicylaldehyde (Auwers and Burger 1904 37 3931) was condensed with malonic acid as usual to obtain 6-bromo-coumarin-3-carboxylic acid (Pandya and Pandya, Proc. Ind. Acad. Sci., 1933 18 A, 164).

The acid was esterified by Fischer-Speier method when the methyl ester was obtained. Crystallisation from methanol gave white needles, m. p. 162°C (Found Br=27.98% $C_{11}H_7O_4Br$ requires Br=28.27%)

Ethyl 6-chloro- or 6-bromo- coumarin-3-carboxylate

5-Chloro-or 5-bromosalicylaldehyde (6 gm) was mixed with diethyl malonate (8 gm) and piperidine (0.2 ml) was added with shaking. The mixture was gently warmed for a few minutes. A solid separated on cooling. It was allowed to stand overnight, filtered, washed with water and a little aqueous ethanol. The following results were obtained:—

(i) Ethyl 6-chloro-coumarin-3-carboxylate, shining white needles m. p. 159°C (lit. 158°C),

(ii) Ethyl 6-bromo-coumarin-3-carboxylate shining white needles m. p. 168°C (lit. 168°-169°C)

6-Chloro- or 6-bromo-coumarin-3-carboxyl chloride

6-Chloro-coumarin-3-carboxylic acid (4 gm) was treated with thionyl chloride (30 ml). A vigorous reaction took place in the cold. The mixture was refluxed on a hot water bath for 1 hour when no more acid fumes were evolved. After cooling petroleum ether (B. P. 40-60°C 100 ml) was added to the clear liquid. The solid which separated was collected and dried.

dry benzene. By adding petroleum ether (B.P 40°-60°C) pale yellow needles of the acid chloride were obtained, m. p 171 C (Found Cl=28.91 $C_{10}H_4O_3Cl_2$ requires Cl=29 22%) The yield was 3.5 gm Buu Ho *et al* (Bull. Soc. Chim., France, 1947 128-136) have also prepared the acid chloride by this method but have not mentioned the melting point and the yield.

6-Bromo-coumarin-3-carboxyl chloride was prepared in the same manner as described by Clinton and Laskowski (J Amer Chem. Soc. 1949 71 3602-67) Pale yellow crystals, m. p. 160° 161°C (lit. 160° 161 C) were obtained

Action of hydrazine hydrate on the esters of 6-chloro-or 6-bromo-coumarin-3-carboxylic acid

No reaction took place when the methyl or the ethyl esters were treated with hydrazine hydrate in an alcoholic solution in the cold. The esters were recovered unchanged on dilution with water

Ethyl 6-chloro-coumarin 3-carboxylate (2 gm.) was dissolved in ethanol (30 ml) by gentle warming and 60 % hydrazine hydrate (3 ml) was added. The yellowish solution was refluxed on a hot water bath for 30 minutes and then cooled in ice. A white solid separated which was filtered, washed with hot ethyl acetate and crystallised from ethanol. White crystals, m. p. 152° C were obtained. Mixed melting point with an authentic specimen of malonic dihydrazide did not show any depression.

The filtrate in the above was evaporated to dryness and the yellow residue was crystallised from hot ethyl acetate, when lemon yellow fibrous needles, m. p. 282° 284°C, were obtained. This was dichloro-salazine. (Found C=54.32%, H=3.05 %, Cl=22.85% $C_{14}H_1O_4N Cl_2$ requires C=54.37% H=3.23%, Cl=22.97%)

When ethyl 6-bromo-coumarin 3-carboxylate was treated under identical conditions, malonic dihydrazide and dibromo-salazine, m. p. 304 C (Found C=41 43%, H=2.7 % Br=40.02% $C_{14}H_1O_2N_2Br$ requires C=42.21 %, H=2.5% Br=40.20 %) were produced.

Similar observations were made when the methyl esters of these acids were used.

By the action of hydrazine hydrate on the acid chlorides of these halogenated acids, dichloro-or dibromo-salazine was obtained.

THORACIC MORPHOLOGY OF *DACUS DIVERSUS* COQ. (TRYPETIDAE : DIPTERA)

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INTRODUCTION

This paper is in continuation of author's earlier two papers† on the morphology of the fruitfly *D. diversus* Coq.

THE THORAX (Figs. 1, 2 & 3)

The three segments of the thorax form a composite structure, with the prothorax and metathorax almost vestigial and the mesothorax greatly developed. The thorax in male is 2.09 mm long and 1.63 mm wide, but in female it is 2.32 mm long and 1.86 mm wide.

The Prothorax.—It lies almost concealed dorsally due to the greatly arched anterior part of the mesothorax and comprises an extremely narrow pronotum (PNM), the lateral pleurites and the ventral sternal region. The propleuron is divided into anterior episternum (ESM_1) and posterior epimeron (EPN_1) by the pleural suture (PLS_1) above the coxa coxa (CX_1) which forms the pleural apophysis internally. The prosternum is distinguished into presternum (*PST*), the cordate basisternum (BST_1) and the triangular furcasternum (FST_1). The spinasternum is not marked.

The Mesothorax.—Nearly the whole dorsal surface of the thorax is covered by the mesonotum, composed of alinotum and the postnotum, the former being divided into the prescutum (PSTM), the scutum (STM) and the scutellum (STLM). The prescutum is a little less than half the mesonotum and bears a pair of lobe-like prescutal lobe (PREL) along its antero-lateral margin (Snodgrass, 1935 and 'Humeral callus' of systematists). The prescutum continues posteriorly into the scutum as the intrascutal suture (ITSS) (or the 'transverse suture') does not meet in the middle. The scutum is produced laterally into anterior and posterior notal wing processes (ANP & PNP). The scutellum is distinctly marked off from the scutum in front by a scutoscutellar suture (SSS) and conceals posteriorly postnotum of the mesothorax ($PONM_2$) and the metanotum (MNM). The postnotum, a well defined area stretching behind the scutellum, is produced into a postero-lateral plate meeting the posterior part of the epimeron of its side.

Contribution No. 90 from the School of Entomology, St. John's College, Agra.

† Nayar, J. L. 1961. External morphology of the head capsule of the fruitfly *Dacus diversus* Coq. *Agra Univ. J. Res.* (*Sci.*) 10 (1): 117-124; Some observations on the genitalia of *Dacus diversus* Coq. *Agra Univ. J. Res.* (*Sci.*) 10 (1): 125-130.

The mesopleuron is a complicated structure. The pleural suture, (PLS_2) extends upwards a *little* from the middle coxa (CX_2) turns dorsally and fol- lows a horizontal plane upto the *middle* of the mesopleuron and finally takes vertical direction to the base of the wing, where it forms the wing process (WP It divides the pleural area into an anterior episternum (ESM_2) and the posterior epimeron (EPN_2) The episternum is divided by a suture into the upper anepi- sternum ($AESM_2$) and the lower katepisternum ($KESM_2$) The former is further subdivided into an anterior pre-anepisternum ($PAESM_2$) and posterior post-anepisternum ($POAESM_2$) by anepisternal suture (AES) arising from the pleural suture. The epimeron is also divided into two by a horizontal suture into an upper larger region anepimeron ($AEPN_2$) and a small katepimeron below ($KEPN_2$) In front of the wing process lie a small somewhat triangular basalare (BA) and an irregular subalare (SA)

The absence of sternopleural suture makes the mesosternum confluent with the pleural area. Ferris (1950) in *Drosophila* held the whole ventral area as the pre-episternum and regarded it as having been formed by the fusion of the katepisternal areas in the discriminal line Crampton (1925) however described this line as the mid ventral suture while Snodgrass (1935) called it the mid ventral groove and Arora (1956) regards this as the mere fusion of the furcal arms along the midventral region of the sternum. The furcasternum lies on the anterior margin of mesothoracic coxal cavities and bears internally the furca. A similar observation is made by Arora (1953) in *Dipteran* [illegible] The location of furca is indicated by a longitudinal furcal groove in the mid- ventral region of the mesothorax.

The Metathorax —The transverse metanotum (MNM) is separated from the postnotum of the mesothorax by narrow membranous area. Metanotum (MNM) is an extremely reduced area with no distinction of metascutum and metascutellum. The postnotum ($PONM_3$) is reduced. The halteres are attached to the sides of the metanotum. The pleural area is divided into a anterior episternum (ESM_3) and posterior epimeron (EPN_3) by the pleural suture (PLS_3) The coxal cavities are separated by a membranous area. Internal to the coxal cavities lie a transverse bridge, representing the basisternum (BSM_3) The furcasternum (FST_3) is extremely reduced and is represented by a small sclerotised area surrounded by the membrane dividing the coxal cavities. Inter- nally the furcasternum bears well developed furca.

The Thoracic spiracles —A pair of spiracles are situated in a sclerotised area separating the prothorax and the mesothorax and the another pair lie in the halteres The former is the true mesothoracic spiracle (MTS) as referred by Singh and Misra (1955) but erroneously labelled as prothoracic [illegible] Coudere (1935) The latter is the metathoracic spiracle (METS) but [illegible] as mesothoracic by Marchel (1897) and Rübsaamen (1925) The true meso- thoracic spiracle is slit like aperture opening into the atrium, which is [illegible]

opens into the trachea. The metathoracic spiracle is very similar to the mesothoracic but smaller in size.

The Chaetotaxy.—The chaetotaxy of the thorax, important from the systematic point, comprise the following:—

(i) *Humeral bristle* (HB).—On the inner margin of the humeral callus.

(ii) *Notopleural bristles* (NPB).—Two pairs, one below the humeral callus and the other above the wing base.

(iii) *Supra alars.*—Three pairs, one pair of anterior supra-alar (ASA) infront of the wing base and two pairs of post supra-alars (PSA) just below the wing base.

(iv) *Mesopleural bristle* (MPB).—On the episternum of the mesopleural area.

(v) *Pteropleural bristles* (PPB).—Small bristles on the wing base.

(vi) *P-scutellar bristles* (PSB).—Two pairs above the scuto-scutellar suture.

(vii) *Scutellar bristles* (SB).—One pair at the base of the scutellum.

The wings (Figs. 4, 5 and 6) are hyaline. The costal cell, marginal cell, and the area along the costal border extending a little beyond the ending of R_{4+5}, anal vein and the cubito-anal cell are dusted black. The spinous costa (C) continues beyond the apex of the wing and terminates at media. The subcosta (Sc) and the radius (R) have a common stalk at the base, which divides into an anterior subcosta (Sc) and posterior radius (R). The subcosta (Sc) extends upto the stigma and curves upwards before its evanscent end. After separating from the subcosta, the radius almost below the humeral vein divides into an anterior first radial vein (R_1) and posterior radial sector (Rs), the former joining the costa a little beyond the middle of the anterior margin of the wing, while the latter extends as a single vein for a short distance only and then splits up into two branches each reaching the apex of the wing. Of these, the anterior represents the R_{2+3} and the other the R_{4+5}. The veins R_1 and R_{4+5} are spinous throughout their lengths.

The media (M), the cubitus (Cu) and the anal (A) all have a common stalk at the base but Miyake (1919) reported the fusion of only media (M) and cubitus (Cu) in *Dacus tsuneonis* Miyake. The common stalk divides into two: the anterior as the media+cubitus (M+Cu) and posterior as the anal (A). Immediately after the separation from the common stalk slight before and below the humeral cross-vein, the anterior takes a sharp curve upwards and extends to the apex of the wing as the media (M_{1+2}), while the posterior representing the cubitus (Cu) extends for some distance before splitting up into two Cu_1 and Cu_2. Above and slightly posterior to the bifurcation of the cubitus into two branches, a very thick branch of the media representing M_3 descends down from

the media and meets the cubitus$_1$ (Cu_1) After meeting M_3 and Cu_1 run toge-ther as M_3+Cu_1 to the middle of the posterior margin of the wing. Before reaching the wing margin it receives another very prominent cross-vein, the median transverse (m t) M_3 was considered as M-Cu cross-vein by Miyake (1919) but on the other hand Benjamin (1934) and Esaig (1954) regard this vein as M_3 The Cu_2 also suffers from the lack of unanimity of opinion regard-ing its true nature. Bezzi (1913) described it as the anal cross-vein, whereas Miyake (1919) Benjamin (1934) and Esaig (1954) were definitely of the view that this vein represents the second branch of the cubitus. Besides the longitudi-nal veins, there are three distinct cross veins viz.—(i) humeral cross-vein (h) between the costa and the subcosta in the first basal quarter of the wing (ii) the radio-medial cross-vein (r m) between R_{4+5} and M_{1+2} and (iii) median transverse vein (m-t) between M_{1+2} and M_3+Cu_1 The cross-veins divide the surface of the wing into five closed cells and eight open cells. The closed cells include (i) first basal costal cell (BCC) (ii) first basal cell (1st BC) (iii) second basal cell (2nd BC) (iv) discal cell (DC) and the (v) cubito-anal cell (Cu Ac) while the open cells are (i) distal costal cell (DCC) (ii) subcostal cell (ScC) (iii) stigma (Stg) (iv) marginal cell (MC) (v) submarginal cell (SMC) (vi) first posterior cell (1st PC) (vii) second posterior cell (2nd PC) and the (viii) third posterior cell (3rd PC) The basal posterior margin is broken up into distinct alula (AL) and axillary lobe (AXL) The former is about one-half in area than the latter The alula is fringed by long bristles and its posterior edge continues as the axillary cord (AXC) which is joined on to the alinotum.

The wing articulation.—(Fig 6) The wing is articulated to the meso-scutum by a membranous base supported by the axillary sclerites, which have not so far been described in Trypetidae There are three axillaries in addition to a humeral plate (HP) at the base of costa (C) and the small tegula (tg) bear-ing spines at the base of the wing The first axillary (1Ax) which articulates with the anterior notal wing process (ANP) is quadrangular with its ante-rior distal angle produced into a long obtusely pointed process stopping short of the common stalk of Sc+R The second axillary (2Ax) is triangular and is attached to the outer margin of the first axillary One of its processes ends at the base of Sc+R. The third axillary (3Ax) is again quadrangular pro-mally articulating with the posterior notal wing process (PNP) and dis-tally it supports the common stalk of M+Cu+A. The median plates are not recog-nised.

The Halteres —The halteres are clavate, spinous and light brown and are attached to the sides of the extremely reduced metanotum in well developed sockets (SOC) Nothing is known in detail of their structure in Trypetidae but Ferris (1950) described them in *Drosophila*. The haltere is a small struc-ture of three distinct regions, a basal scabellum (SBM) narrow elongated pedicel (PED) and a swollen distal capitellum (CTM) Special sensoria (SEN) are

present on the dorsal side of the scabellum, and are arranged in six rows in addition to a row on either lateral side of the pedicel The capitellum is clothed by widely distributed setae or bristles in addition to the minute pubescence.

Legs (Figs. 7 8 and 9)—These are moderately long, slender setose and black. The mid legs are the longest and are approximated to the hind legs. The fore legs are far removed. The coxa (CX) is short, stout and quadrangular and the trochanter (TRC) still shorter and thinner than the former The femur (FM) is extremely elongated and almost as long as tibia (TB) The tibial spurs are present in the middle leg only Tarsus (TA) is as long as the tibia, with the metatarsus (MTA) half the latter and four times as long as thick. The rest of the tarsal segments are sub-equal. Each tarsal segment bears three to five prominent setae. The pretarsus (PTA) bears a pair of prominent stout claws (CL) pulvilli (PLV) unguitractor (UNT) empodium (EMP) and unguifer (UNF) The claws are articulated to the median distal end of the last tarsomere called the unguifer the arolium being absent. The pulvillus arises laterally from the basipulvillus or auxillary plate (AUXP) Transversely sculptured well defined unguitractor lies ventrally proximal to the auxillary plate. Distally it bears an extremely long, slender spine the empodium and proximally carries the retractor of the claws or the flexor tendon (FLXT)

ACKNOWLEDGEMENTS

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REFERENCES

1 Arora, G L. 1953. The external morphology of *Diprion pini* (L.) *Res. Bull. E. Panj. Univ.*, 25 1 23.

2. Arora G L. 1956. The relationship of Symphyta (Hymenoptera) to other orders of Insect on the basis of adult external morphology *Res. Bull. E. Panj Univ* 90 85-119

3 Benjamin F H. 1934 Description of the native Trypet flies with notes on their habit. *Tech. Bull. U.S Dept. Agric.* Washington, D. C. No. 401

4. Bezzi, M. 1913 Indian Trypaneids (Fruitfly) in the collection of Indian museum, Calcutta. *Mem. Indian Mus.*, Calcutta, 3 53-174

5. Coudere, J 1933. Etude monographique de la cecidomyii de la violette. *Bull. Soc Hist. Nat. Toulouse* 63 (2) 193-279.

6. Crampton, G C. 1925. A phylogenetic study of the thoracic sclerites of the non-tipuloid nematocerous Diptera. *Ann. ent. Soc. America*, 18 49-67

7 Essig, E. O 1954. College Entomology The MacMillan ComCpany New York

8. Ferris, G F 19 0. External morphology of *Drosophila* given in the biology of *Dro...* Edited by M. Demerec N Y John Wiley & Sons, Chapman & Hall Ltd, London

9 Marchel P 1897 Des cecidomyies cereals t leurs parasites. *Ann. Soc. ent. Fr.* 6 : 1 105

10. Miyake T 1919 *Bull. Imperial Central Agric. Experiment. Sta. Japan*, 2. No. –

11 Rübsaamen, E H & Hedicke H. 1925 Die Cecidomyiden und ihre Cecidien Stuttgart pp 1 103

12. Singh, S & Misra, S K. 1955 Morphology of the paddy gall midge *Pachydiplosis oryzae* Manl. (Itonididae = Cecidomyiidae Diptera) *Agra Univ. J Res.* (Sci.) 4 (2) : 427-452.

13 Snodgrass, R E. 1935. Principles of Insect Morphology McGraw Hill Book Co, New York and London.

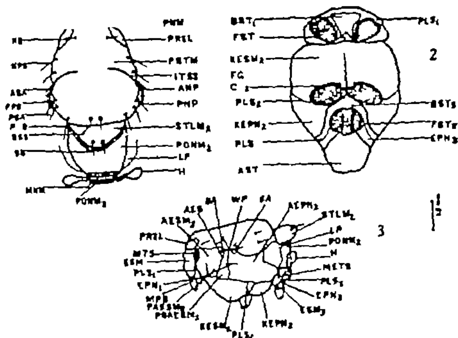


Fig. 1 Dorsal view of the thorax.

Fig. 2. Ventral view f the thorax

Fig. 3. Lateral view of the thorax.

$AESM_2$, anepisternum₂ $AEPN_2$, anepimeron ; ANP anterior notal wing process; ASA anterior supra alar bristle BA, basalare; BSM_2, basisternum; EPN epimeron ; ESM_1 episternum₁; EPN_2, epimeron₂; ESM_2 episternum₂; FST furcasternum; FST_2 furca-sternum₂ FG, furcal groove; H, haltere HB humeral bristle; ITSS, intra sc tal suture $KEPN_2$, katepimeron₂; $KESM_2$, katepisternum₂; LP lateral plate; METS, metathoracic spiracle MNM metanotum MPB mesopleural bristle; MTS, mesothoracic spiracle NPB notopleural bristle $POAESM_2$, post-anepisternum₂; $PAESM_2$, preanepisternum₂ PNM, prono um PNP posterior notal wing process; PLS_1 pleural suture₁; PLS_2, pleural suture₂; PLS_3, pleural suture $PONM_2$ post-notum₂ $PONM_3$, post-notum₃; PPB pteropleural bristle PSA, Posterior supra alar; PSB, prescutellar bristle; PST pre-sternum PREL, prescutal lobe; PSTM prescutum S.A. Subalare SB, scutellar bristle; $STLM_2$, scutellum₂ STM, scutum.

Fig 4 Haltere
Fig 5. Wing
Fig. 6. Attachment of the wing

A. analvein A+Cu_2 annal+cubital$_2$; AL. alul 1AX. first axillary 2AX. second axillary 3AX. third axillary ANP anterior notal wing process; AXL. axillary lobe; 1st BC first basal cell 2nd BC. second basal cell; BCC. basal costal cell; C. costa; Cu_1 cubitus$_1$ Cu_2 cubitus$_2$ Cu-AC. cubitoanal cell CTM. capitellum DC. discal cell, DCC. discal costal cell M_1+Cu_1 media$_1$+cubitus$_1$ M_{1+2} media$_{1+2}$ M_3 media$_3$ MC. marginal cell M3+Cu1; PED. pedicel 1st PC. first M+Cu+A. media+cubitus+anal m-t median transverse vein; PED. pedicel 1st PC. first posterior cell 2nd PC. second posterior cell 3rd PC. third posterior cell PNP posterior notal wing process R. radius, R_1 radius$_1$ R_{2+3} radius$_{2+3}$, R_{4+5} Radius$_{4+5}$ RS radial sector rm. radio-medial cross vein SBM. scabellum SC. subcosta ScC. subcostal cell SCT. scutum SMC. submarginal cell Stg stigma SOC. socket

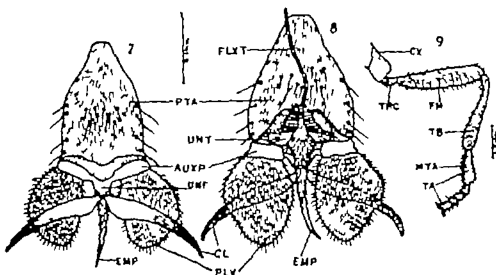


Fig. 7. Pretarsus (prothoracic leg) Dorsal view

Fig. 8. Pretarsus (prothoracic leg) ventral view

Fig. 9. Metathoracic leg.

AUXP auxillary plate; CL claw; CX coxa; EMP empodium; FLXT flexor tendon; FM femur; PTA pretarsus; TA tarsus; TB tibia; TRO trochanter; UNF unguifer; UNT unguitractor

EXTERNAL MORPHOLOGY OF THE THORAX OF *SPHRACEPHALA HEARSEYANA* WESTW (DIOPSIDAE DIPTERA)

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INTRODUCTION

This paper deals with descriptive morphology of the thorax of common Diopsid fly *Sphracephala hearseyana* Westw The morphology of the head capsule and the wing venation have been worked out by Nayar and Tandon† The studies on other systems will follow in subsequent papers

Our thanks are due to Dr T Singh, Professor of Zoology and Entomology for facilities for work.

MATERIAL AND METHOD

The Diopsid flies fo this work, collected from the walls of the bath-room in Lloyd's Hostel St. John's College Agra, during the months of November and December 1960 were decolorised by chlorine fumes, upgraded and dissected in Canada balsam for detailed study Diagrams were drawn with the help of the camera lucida.

The Thorax (Figs. 1 2 and 3)

The thorax is a composite structure formed by the fusion of prothorax, mesothorax and metathorax. The mesothorax forms the major portion of the thorax whereas the prothorax is poorly developed and the metathorax almost reduced to a mere vestige. The whole structure in the male is 3 50 mm long and 3 00 mm. wide, while in the female it is 3 70 mm. long and 3 00 mm. wide.

The prothorax—It constitutes a narrow collar infront of the mesothorax The pleural and sternal regions are fairly well-developed while the tergum is represented by a simple transverse sclerotised plate. The protergum (PNM) lies hidden beneath the prescutum of the mesothorax and is produced dorsally into an elevated concavity for the reception of the knob-like process of the occipital region of the head. The propleuron is divided into an anterior episternum (EPS_1) and posterior epimeron (EPM_1) by the pleural suture (PLS_1)

Contribution No. 96 from the School of Entomology St John's College Agra.

† Nayar J L. and Santosh K. Tandon. A note on the wing venation of *Sphracephala hearseyana* Westw *Agra Univ. J Res.* (Sci.), 11 (1) 113-116 External morphology of the head capsule of *Sphracephala hearseyana* Westw *Ibid.* 11 (1) 131 138.

arising from the base of the coxa, (CX_1) forming the pleural ridge internally for insertion of the muscles. The prosternum is a composite structure comprising a eusternum only and no spinasternum. A pair of prominent sternal apophysial pits (AP) are present laterally in the sternum posterior to the procoxae, connected by the sternocostal suture (SCS) which divides the eusternum into a presutural area, the basisternum (BSM_1) and a transverse posterior portion, the sternellum (SL) The anterior part of the eusternum, called the presternum (PSM) is cut off from the basisternum (BSM_1) by a presternal suture (PRS) running between the bases of the procoxae. The presternum (PSM) is distinctly divided into a median sclerotised portion and lateral triangular membranous areas. Infront of the presternum lies the cervical membrane (CM) bearing laterally two pairs of small cervical sclerites (CS)

The mesothorax—The mesothorax, forming the major portion of the thorax, is a highly complicated structure and is projected anteriorly to cover the prothorax. The alinotum comprises the prescutum (PSTM) the scutum (STM) and the scutellum (STLM) The prescutum (PSTM) is twice as wide as long and is produced anterolaterally into well-marked lobes called the prescutal lobes (PREL) by Snodgrass (1935) or the humeral calli" of systematists. The scutum is not completely separated from the prescutum infront since the intrascutal suture (ITSS) or the transverse suture of Diptera is incomplete in the centre. Laterally the scutum is produced into an anterior and posterior notal wing processes (ANP and PNP) The scutoscutellar suture (SSS) completely separates the scutum from the scutellum (STLM) posteriorly Scutellum (STLM) is a fairly large subtriangular area projecting posteriorly over the postnotum (PONM) and the metanotum (MNM) Posteriorly the scutellum is produced into two prominent scutellar processes (STLMP) bearing scutellar bristles apically The postnotum (PONM) lies vertically just beneath the scutellum and is produced laterally into small lateral plates (LP) meeting the ... of its side. Each lateral plate (LP) bears prominent triagular processes.

The pleuron and the sternum are not separated from one another. The pleural region is divided by the pleural suture (PLS_2) into two areas the anterior mesepisternum and osterior mesepimeron The pleural suture after arising posteriorly from the rim of the coxal cavity follows a tortuous course. First it runs vertically for a short distance takes a horizontal position and finally turns vertically upwards to the base of the wing The mesepisternum is partially divided by a suture into two regions, an anterior anepisternum (AEPS) and the posterior katepisternum (KEPS) Similarly the mesepimeron is divided into an upper anepimeron ($AEPM_2$) and a lower small area, the katepimeron ($KEPM_2$) by a suture arising from the pleural suture (PLS) at the point where the latter takes a horizontal course The anepimeron ... postero-dorsally with the lateral plate of the pronotum. Anteriorly an ... fectly separated lobe of mesanepisternum ($AEPS_2$) below the wing base ... basalare (BA) and posteriorly two distinctly separated subalare sclerites (SA) ...

SA_2) are recognised in the mesanepimeron ($AEPN_2$) Subalare (SA_2) is almost double the length of subalare (SA_1)

As already pointed out, the mesosternum is not distinguishable from the mesopleuron as the sternopleural suture is lacking There seems to be a great deal of lack of uniformity of opinion regarding the nature of the sternal region of Diptera Some authorities like Ferris (1950) believe that the whole area is the fused episternal portions, while others, particularly Snodgrass (1935) suspect that the sternal groove is represented internally by a median ridge formed by the extension of the furcal base anteriorly Lowne (1892) reported the absence of a distinct furcasternum in *Calliphora* so it is not possible to say as to how much of the mesosternal portion has become inflected to form the ridge On the contrary in *Sphaerocephala beersyana* Westw a well-marked furcasternum (FS_2) is present which indicates that the mesosternum is very likely composed of portions of sternum, precoxal bridge and parts of episterna. The furcasternum (FS_2) lies as a small piece between the coxal cavities (CX_2) from where arises the furca internally The midcoxal cavities are almost confluent.

The metathorax—The metanotum (MNM) is an extremely reduced transverse area behind the postnotum (PONM) There is no division of it into metascutum and metascutellum. Laterally the metanotum bears the halteres (H) The metapleuron is distinctly divided by the pleural suture (PLS_3) into an anterior episternum (EPS_3) and the posterior epimeron (EPN_3) The metasternum is divided into an anterior extremely wide area the basisternum (BSM_3) and a small part the furcasternum (FS_3) infront of the hind coxae (CX_3) The furcal groove (Fu_3) runs in the middle of the basisternum (BSM_3) bearing internally the furca. Snodgrass (1935) regards the basisternum (BSM_3) as the region of postcoxalia of the mesothorax, the precoxalia of the metathorax and the metathoracic basisternum. Postero-mesial border of the metacoxal cavities is formed by the first sternite of the abdomen (IS)

The thoracic spiracles—Two pairs of thoracic spiracles are recognised, one in the membranous area of the proepimeron and the other also similarly placed in a region below the base of the haltere. The former is the mesothoracic spiracle (SP_2) and the latter metathoracic spiracle (SP_3) Each spiracle is a slit like aperture on a stigmal plate surrounded by the membranous area. The metathoracic spiracle is about twice the size of the mesothoracic spiracle.

Thoracic chaetotaxy (Fig 1)—The chaetotaxy of the thorax comprise the following —

1 *Anterior supra alar bristle* (ASAB)—A pair of bristles infront of the wing base

2. *Presutural bristles* (PSB)—A pair of small bristles infront of the transverse suture.

3 *Prescutellar bristle* (PSB)—A pair of long bristles on the postero-lateral margin of the scutum.

4 *Scutellar bristle* (SB)—A pair of very long bristles borne apically on the scutellar processes.

The legs—(Figs 4 4a 5 6, 7 and 8)—The legs are long, slender spurs and black. The meso-and metathoracic legs lie close to each other while the prothoracic are far removed infront. Each leg comprises a coxa (CX), trochanter (TRC) femur (FM) tibia (TB) and five segmented tarsus (TA). The meso-and the metathoracic legs are almost of the same size with slender femora and tibia. The tarsus of the mesothoracic leg is as long as the tibia and metatarsus (MTA) about one-third the latter In the hind leg the tarsus is slightly longer than the tibia and the metatarsus a little more than half as long as the latter The prothoracic leg bears a very long coxa and highly swollen femur On the inner surface of the femur there are present two rows of pegs, the outer row bearing 26 pegs and the inner row 23 pegs. The function of these pegs seems to be to produce sound by rubbing against the inner surface of the tibia which is kept folded on it while sitting The inner surface of the tibia is roughened The pretarsus (PTA) (Figs. 7 and 8) comprises a pair of prominent claws (CL) pulvilli (PLV) ungultractor (UNT) empodium (EMP) and unguifer (UNF) The claws are attached dorsally to the median terminal end of the pretarsus called the unguifer The finger-shaped pulvilli arise from the auxillary plates (AXP) A well-defined transversely sculptured unguitractor plate lies ventrally a little anterior to the auxillary plates. On its proximal end is inserted the flexor tendon or the retractor of the claws (RCL) Distally it bears a long slender spine, the empodium (EMP)

Summary

The thorax is a composite structure with the mesothorax well developed, the prothorax poorly marked and the metathorax almost vestigeal. A detailed account of the pleural and sternal areas is given. The thoracic chaetotaxy and legs were studied.

References

1 Ferris, G. F 1950. External morphology of *Drosophila* given in the biology of Drosophila J Wiley & Sons, New York, (Chapman & Hall) London.

2. Lowne B. T 1892. The Anatomy Physiology Morphology and Development of Blowfly Vol. I, 350 pp.

3. Snodgrass, R. E. 1935 Principles of Insect Morphology New York & London (McGraw Hill Book Co.)

Fig. 1. Dorsal view of the thorax.
ANP anterior notal wing process; ASAB. anterior supra alar bristle; H. haltere; ITSS intra scutal suture MNM. metanotum PNM. pronotum; PNP posterior notal wing process; PONM. postnotum; PREL. prescutal lobe PRSB. prescutellar bristle PSB. presutural bristle; PSTM. prescutum; SB. scutellar bristle; STLM. scutellum; STLMP scutellar process; STM. scutum.

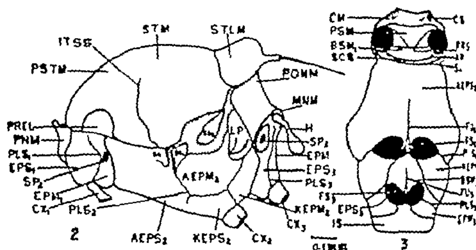


Fig. 2. Lateral view of the thorax.

Fig. 3. Ventral view of the thorax.

AEPM$_2$, anepimeron$_2$; AEPS$_2$ anepisternum$_2$; AP apophysial pit; BL basalar; BSM$_1$ basisternum$_1$; BSM$_2$, basisternum$_2$; CM. cervical membrane; CL cervical sclerite; CX$_1$ coxa$_1$; CX$_2$, coxa$_2$; CX$_3$ coxa$_3$; EPM$_1$ epimeron$_1$; EPM$_2$ epimeron$_2$; EPM$_3$ epimeron$_3$; EPS$_1$ episternum$_1$; EPS$_2$ episternum$_2$; EPS$_3$ episternum$_3$; FU$_2$ furcal groove or sternal groove$_2$; FS$_2$, furca sternum$_2$; FS$_3$, furcasternum$_3$; FU$_3$ furcal groove or sternal groove$_3$; H haltere; IS first abdominal sternite; ITSS intrascutal suture; KEPM$_2$ katepimeron$_2$; KEPS$_2$ katepisternum$_2$; LP lateral plate; MNM mentanotum; PLS$_1$ pleural suture$_1$; PLS$_2$ pleural suture$_2$; PLS$_3$ pleural suture$_3$; PNM. pronotum; PONM. postnotum; PREL prealar lobe; PRS. presternal suture; PSM presternum; PSTM. prescutum; SA$_2$ subalare$_2$; SL. sternellum; SCS. sternocostal suture; SP$_2$, mesothoracic spiracle$_2$; SP$_3$, metathoracic spiracle$_3$; STM. scutum; STLM. scutellum

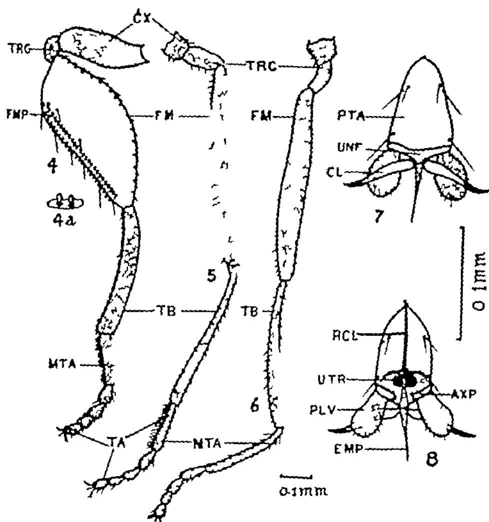


Fig 4. Prothoracic leg.
Fig. 4a. Enlarged view of the femoral pegs of the prothoracic leg
Fig. 5. Mesothoracic leg
Fig. 6. Metathoracic leg
Fig. 7 Dorsal view of pretarsus
Fig. 8. Ventral view of pretarsus.

AXP auxillary plate; CL claw; CX. coxa; EMP empodium; FM femur; FMP femoral pegs; MTA. metatarsus; PLV pulvillus PTA. pretarsus; RCL. retractor of claws TA. tarsus TB tibia TRC trochanter; UNF unguifer; UTR. unguitractor

THE ENDOSKELETON OF *BAGARIUS BAGARIUS* (HAM.)
Part II—The Vertebral Column, Median Fins and Appendicular Skeleton

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INTRODUCTION

In a previous contribution the skull of *Bagarius bagarius* has been dealt with and the present work is devoted to the rest of endoskeleton of the fish.

Besides McMurrich (1884) Sarbahi (1931) Dharmarajan (1936) Chapman (1941 44a & 44b) Phillips (1942) Joshi & Bal (1953) and Newar (1954) on the complete endoskeleton of different fishes references may be made to Elms (1870-71) Ramanujam (1929) Mookerjee Mitra & Mazumdar (1940) Ford (1941) Mookerjee, Ganguly & Mukerji (1950) and Kamel (1956) on vertebral column Howes (1887) Goodrich (1906) Whitehouse (1910, 1918) Graham (1936) Hollister (1940) Eaton (1945) and Affleck (1950) on fins and appendicular skeleton

My thanks are due to Dr B M. Sinha for the guidance during the course of work.

THE VERTEBRAL COLUMN

The vertebral column consists of fortyone vertebrae of which twenty are trunk vertebrae and remaining caudal vertebrae

The trunk Vertebrae

A typical *trunk Vertebra* (I-2) has an amphicoelous centrum, each face of the centrum being deeply concave. The edges of the adjacent centra are united by ligamentous tissue and the space between them is filled with the remains of notochordal tissue. The centrum has the usual median-dorsal, median ventral, dorso-lateral and ventro-lateral grooves. From the antero-lateral borders of the median dorsal groove arises a backwardly directed neural arch, which is produced distally into a laterally compressed neural spine. The base of the neural arch is thickened and it bears a pair of anterior zygapophyses in front and a pair of posterior zygapophyses behind. The anterior zygapophyses of a vertebra oppose the posterior zygapophyses of the preceding one enclosing between them apertures for the spinal nerves. From the ventro-lateral aspect of the centrum are given off stout parapophyses, which run downwards and backwards and bear ribs at their distal ends.

The *anterior vertebrae* of this region are very much modified in connection with the Weberian ossicles. The second third and fourth vertebrae are intimately connected to form the complex vertebra (I 1) to which is ankylosed the

first vertebra in the form of a cylindrical piece. The neural spines of the complex vertebra form a laterally compressed neural plate, which increases in height gradually towards the posterior end. It articulates in front with the process of the supraoccipital and behind with the neural arch of fifth vertebra. The parapophyses on the second and third vertebrae are wanting, while on the fourth are broad flattened and directed upwards forming a semicircular inverted arch. Each parapophysis arises from the full length of complex vertebra and is folded distally into an anterior and posterior division. The *anterior division* is [illegible] and directed outwards articulates with the posttemporal. Its front margin is directed downwards and backwards forming a pocket into which is received the anterior part of air bladder. The *posterior division* is thin and directed backwards articulates with the transverse process of fifth vertebra. Below the complex vertebra is a pair of laminated processes, each of which encloses the [illegible] paravertebralis between it and the parapophysis of its side. Below the complex vertebra has a pair of lateral ossifications internal to the laminated processes, which bound between them the canal for the dorsal aorta. The fifth vertebra is secondarily fused with the complex vertebra. The posterior face of this vertebra is free and bears the concave centrum and posterior zygapophyses. Its neural spine is bifid and its parapophyses are without ribs.

The centra of the vertebrae (I 3 & 4) from six to eleven elongate progressively towards the posterior end. Their neural spines are poorly developed and crest-shaped while their parapophyses become gradually directed downwards. The neural spines of sixth to ninth vertebra bear grooves along their anterior faces for the radials of dorsal fin. The centra of vertebrae from twelve to twenty (I 2 5 & 6) are nearly uniform in length. Their neural spines are crest-shaped, but gradually incline backwards and become pointed. Their parapophyses are directed downwards and are joined by transverse bridges. There is one transverse bridge in each vertebra except the last one which is furnished with two such bridges (I-6)

The Caudal Vertebrae

A typical *caudal vertebra* (I 7 & 8) resembles the typical trunk vertebra except for minor details. Its neural spine is more elongated and much [illegible] backwardly directed From the antero-lateral margin of the median [illegible] groove arises the haemal arch, which is produced into a backwardly directed haemal spine. The base of the haemal arch is thickened and is produced in front into spine-like antero-ventral processes Similar postero-ventral processes directed backwards, project from the posterior face of the centrum.

The last seven caudal vertebrae are modified in relation with the caudal fin Their neural and haemal arches get gradually reduced, while [illegible] become progressively elongated and stout to support the fin The last [illegible] vertebra is without the posterior zygapophyses and posterior ventral [illegible] and is produced behind into an upturned urostyle Articulating [illegible]

caudal vertebra is a free radial above the urostyle and five laterally flattened hypurals below it. The five hypurals are separated by a deep notch into two upper and three lower for the epichordal and hypochordal lobes of the caudal fin respectively. The neural spines of the last but one and five preceding caudal vertebrae work as epurals and their haemal spines as hypurals.

Associated with the distal ends of parapophyses of the trunk vertebrae are fifteen pairs of *ribs*. Each is a slender rod directed downwards and backwards between the muscle segments. The proximal end of a rib is expanded and articulates with its parapophysis, while the distal end is pointed and ends freely in the musculature. The anterior ribs are better developed than the posterior ones.

The Median Fins

A second dorsal fin is present as an adipose fin, but is without fin rays. The skeletal elements of the median fins are the radials and fin rays. Each radial is typically made of proximal, mesial and distal components. The fin rays are paired lepidotrichia, which are closely approximated together and appear as single ray. At the proximal end of the ray the two lepidotrichia enclose between them the distal radial and at their distal ends they are provided with unjoined horny actinotrichia.

The Dorsal Fin

The *dorsal fin* (II 1) is well developed and bears seven rays seated on seven radials. The radials get distally fused and it is difficult to make out their mesial and distal components. The proximal component is large and more or less dagger-shaped. At the distal end it gives off two processes and at the proximal end it is pointed. The component is provided with four longitudinal ridges, a usually developed anterior, two similar lateral and a well developed posterior ridge. The first and second radials fuse with one another by their opposing ridges throughout the length, while rest of the radials unite by their distal parts only. The lower end of the first two fused radials is received in a groove on the sixth vertebra and of the third, fourth and fifth in the grooves on neural spines of the seventh, eighth and ninth vertebrae. The lower end of the sixth radial lies over the ninth vertebra, while the short seventh radial is applied to the posterior ridge of the sixth radial.

The *first radial* (II 2) is strongly developed. It is deeply grooved along its anterior side and from its upper surface are given off two backwardly directed processes. Its posterior ridge combines with the anterior ridge of the second radial and the two together are produced above into a laterally compressed keel which supports a saddle like osseous structure. This saddle-like structure probably represents the aborted fin ray of the first radial. The second radial is also produced above into a pair of backwardly directed processes which fuse with the similar processes of first radial.

The fin is composed of a spine and six fin rays The spine is well developed and is produced distally into a non-osseous filament, which extends much beyond the fin. The other rays are of the usual type, segmented and branched.

The Anal Fin

The *anal fin* (II 3) consists of thirteen segmented rays supported on the same number of radials. The proximal segment is long and spine-like and gradually reduces in size from the first to last radial Its apex lies between the haemal spines of the two adjacent caudal vertebrae and its base fuses with its medial segment. The distal segment is a rounded nodule, which lies in a depression on the anterior face of succeeding radial. The radials are supported on seven caudal vertebrae from sixteenth to twentysecond. As the number of fin rays is thirteen and the number of vertebrae supporting them seven, more than one radial is present in relation with a single haemal spine. There are two radials in each of the interspace between the haemal spines of fifteenth and sixteenth, sixteenth and seventeenth, eighteenth and ninteenth, and twenty first and twenty second vertebrae one radial in each space between the haemal spines of seventeenth and eighteenth and ninteenth and twentieth vertebrae and three radials in between the haemal spines of twentieth and twentyfirst vertebrae. The first feeble ray and the two following ones are unbranched, while the remaining are branched In front of the first ray and applied to its anterior face is an additional poorly formed ray

The Caudal Fin

The *caudal fin* (III) is vertically expanded. Its dorsal lobe consists of eight rays, while the ventral lobe is composed of nine rays, which run the full length of the fin. The rays are segmented and branched except the uppermost and lowermost rays, which are unbranched and extend beyond the fin as filaments Besides, there are twelve small rays at the base of the upper lobe and fourteen such rays at the base of the lower lobe which are unbranched and unsegmented The caudal fin is deeply concave distally Its dorsal lobe is supported on the neural spines of last but one and five preceding vertebrae free radial urostyle and first two hypurals. The neural spines support ten rays, the free radial two rays, the urostyle one ray and the hypurals seven rays. The ventral lobe is likewise supported by the haemal spines of last but one and five preceding vertebrae and remaining hypurals. The haemal spines support fifteen rays and hypurals eight rays. Owing to the vertebral axis turned upwards the epichordal lobe is reduced and bears twelve rays, while the hypochordal lobe carries the rest of the rays Externally thus a symmetry is developed and the tail is homocercal

THE APPENDICULAR SKELETON

The Pectoral Girdle

The *pectoral girdle* (IV 2) lies behind the branchial cavity and is made up of two identical halves, each distinguished into a primary and a secondary part

The primary part is reduced and is closely applied to the lower side of the secondary part. It is composed of the scapula coracoid and mesocoracoid. The secondary part is prominent and includes the cleithrum and posttemporal.

The *scapula* is triangular in form and is placed between the two limbs of the cleithrum. Its upper limb articulates with the dorsal limb of cleithrum, the anterior with coracoid and the posterior together with the extension from the coracoid forms the articular surface for the fin. The bone is perforated with the scapular foramen.

The *coracoid* is well developed and lies immediatly below the ventral limb of cleithrum. It fuses with the cleithrum by its anterior face and interdigitates distally with the coracoid from the other half. The coracoid is produced behind into a process, which is directed downwards and forwards and articulates with the ventral limb of cleithrum. The joint between the bone and this process extends in the form of articular surface for the fin.

The *mesocoracoid* is a vertically directed splint like bone, which articulates with the dorsal limb of the cleithrum at the upper end and the scapula below. At the lower end it is also connected with the coracoid.

The *cleithrum* is a curved prominent bone distinguished into a dorsal and a ventral limb. The dorsal limb is more prominent and running vertically up terminates in the notch of posttemporal. The ventral limb is less prominent and extends beneath the head to articulate with the cleithrum of other side. In the angle between the two limbs of the cleithrum is a depression in which glides the condylar head of the spine of pectoral fin.

The *posttemporal* (IV 1) acquires a secondary association with the cranium. It has a main body produced into two processes, the superior and inferior. The body carries a deep notch, in which is received the dorsal limb of cleithrum. The superior process articulates with the supraoccipital, pterotic epiotic and complex vertebra while the inferior process abuts on the facet presented by the basioccipital and exoccipital.

The Pectoral Fin

The *pectoral fin* (IV 2) has a spine and thirteen fin rays. The spine is strong, being serrated at its anterior edge and produced into teeth along the posterior edge. It is produced into a long non-osseous filament, which runs much beyond the fin. The spine articulates with the girdle by a double head, the more prominent condylar head moves in the depression of cleithrum and the other articulates on the articular surface provided by the scapula and coracoid. In the cartilage between the girdle and pectoral fin are two flattened radials of which the second is large and well developed. The first two rays articulate directly with the cartilage, while the third and fourth rays lie over

the first radial. The fifth ray articulates in the interspace between the first and second radial and the remaining seven rays are supported over the second radial.

The Pelvic Girdle

The *pelvic girdle* (IV-3) consists of a pair of pelvic bones, embedded in the cartilage. Each pelvic bone is in the form of a flattened plate produced in front into two processes The flattened plate is perforated and fails to join the plate of the other side medially Of the two processes the inner is slightly [illegible] ger and meets a similar process from the other pelvic bone. The posterior end of the bone is convex and with it articulates the fin

The Pelvic Fin

The *pelvic fin* (IV 3) includes six fin rays and an extra supernumerary. The radials are absent and the rays articulate directly with the girdle. The first fin ray is unbranched while the rest are segmented and branched. The supernumerary is a small bony rod attached to the first ray and directed away from the pelvic bone.

SUMMARY

1 The vertebral column consists of fortyone vertebrae of which [illegible] are trunk vertebrae and rest caudal vertebrae The first four vertebrae [illegible] the complex vertebra. Its neural arch is crest-shaped while the [illegible] are in the form of inverted arch. The fifth vertebra gets a secondary [illegible] toin with the complex vertebra The parapophyses of the last nine trunk vertebrae are furnished with transverse bridges.

2 The neural and haemal spines of the last six caudal vertebrae work as epiurals and hypurals The urostyle supports the free radial and five hypurals. Two of the hypurals support the epichordal lobe and the remaining three the hypochordal lobe.

3 The dorsal fin has an emarginate spine and six fin rays supported on the same number of radials. The mesial and distal segments of the radials can not be made out The first and second radials are modified for the [illegible] tion and depression of fin

4 The anal fin includes thirteen rays borne on an equal number of radials. The proximal and distal segments of the radials are distinct, while the [illegible] one gets fused with the former As the number of fin rays is thirteen and the vertebrae supporting them seven more than one radials are borne on [illegible] the haemal spines.

5 The caudal fin is homocercal. The epichordal and hypochordal lobes carry eight and nine complete rays of which the uppermost and lowermost rays are unbranched [illegible] emarginate Besides there are twelve [illegible] rays at the base of [illegible] be and fourteen along the base of the [illegible]

6. The primary part of the pectoral girdle comprises the scapulae coracoids and mesocoracoids, while the secondary part is formed of posttemporals and cleithra. The posttemporals are rigidly fused with the cranium. The pectoral fin has an emarginate spine and twelve fin rays. Two radials lie in between the girdle and the fin

7 The pelvic girdle is formed of two pelvic bones each produced in front into two processes. The pelvic fin has six rays and an additional supernumerary Radials are absent.

References

1 Affleck R. J 1950 : Some points in the function development and evolution of tail in fishes. *Proc Zool. Soc London* CXX : 319-368.

2. Ellis, R. 1870-71 On the osteology of anterior vertebrae in *Doras niger* with comparison of the structure of the dorsal fin in *Doras* and *Balistis* *Proc Boston Soc. Nat. Hist.*, 14 : 3 12

3. Chapman, W M. 1941 : The osteology and relationship of the Isospondylous fish, *Plecoglossus altivelis*. Jour Morph. LXVIII 425-456.

4 Chapman, W M. 1944a. On the osteology and relationship of South American fish, *Aplochiton zebra*. Jour Morph., LXXIV 149-166.

5. Chapman W M. 1944b. : The osteology of the Pacific deep bodied anchovy *Anchoa compressa*. *Jour Morph.* LXXIV 311 329

6. Dharmarajan, M. 1936 : The anatomy of *Otolithes ruber* Part I. The endoskeleton. *Jour Roy Asia Soc Bengal.*, II 1 72.

7 Eaton T H. Jr 1945 Skeletal supports of the median fins of fishes. *Jour Morph.* LXXVI : 193-212.

8 Ford E. 1941 Vertebral variations in teleostean fishes. Part II The herring *Clupea harengus* Jour Marine. Biol. Ass. Plymouth XXV 151 172.

9 Goodrich E. S. 1906 Notes on the development structure and origin of the Median and Paired Fins of fish. *Quart. J Micro Sci.*, 50 333-376

10. Graham S 1936 The tail of fishes. *Proc Zool. Soc London*, 106 595-603

11 Howes G. B. 1887 Skeleton and affinities of paired fins of *Ceratodus*. *Proc Zool. Soc London* 37 3-26.

12. Hollister G. 1940 Caudal skeleton of Bermuda shallow water fishes IV Zoologica. New York, XXV 97 112.

13. Joshi, M. S. & Bal D V 1953 The skeleton of *Coilia dussumieri* Part I Skull Part II Vertebral Column nd appendicular skeleton *Jour Univ Bombay* N S. XXI B 53-63.

14 Mcmurrich, M. 1884 The osteology of *Amiurus catus*. *Proc Canad. Inst. Sci* N S. II 270-310.

15. Kamal A. H. 1956 On the development of the vertebral column in *Gambusia affinis* Bull. Fac. Sci. Egypt Univ XXXV 95-116.

16. Ramanujam S. G. Manawala 1929 The study of the development of the vertebral column of Teleosts as shown in the life history of the Herring *Proc Zool. Soc London*, 99 365-414

17 Mookerjee H.K. Mitra, G N And Mazumdar S. R. 1940 The development of the vertebral column of viviparous Teleost *Lebistes reticulatus*. Jour Morph. LXVII 241 269.

18. Mookerjee, H. K. Ganguly D N and Mukerji, P S 1950 : On the structure of the vertebral column and the Weberian ossicles in Carpmnow *Ezan Lrn*. Proc Zool Soc. Bengal V : 167 182.

19 Newar G 1954 : On the anatomy of *Clarias batra* I Osteology Jour. Morph. XCIV : 551-585.

20 Phillips J B. 1942 : The osteology of the sardine *Sardinops caerulea*. Jour Morph. LXX : 463-500

21 Regan C. T 1910 : Caudal fin of Elopidae and some other Teleostean fishes. Ann. Mag Nat. Hist. Ser B V : 354-358.

22 Sarbahi D S 1931 : The endoskeleton of *Labeo rohita*. Jour Proc. Asia Soc. Bengal XXVIII. 295-347

23 Whitehouse R H. 1910 : Caudal fin of Teleostomi. Proc. Zool. Soc. London, II 590-626.

24 Whitehouse R. H. 1918 : Evol tion of the Caudal Fin of fishes. Rec. Ind. Museum, XV : 135-142.

EXPLANATION OF PLATE

Fig (1) Front view of complex vertebra ; Fig. (2) Side view of vertebrae from the posterior trunk region Fig (3) Side view of vertebrae from middle trunk region Fig (4) Hind view of a vertebra from middle trunk region Fig. (5) Hind view of a vertebra from posterior trunk region ; Fig (6) Hind view of a last trunk vertebra Fig (7) Sid view of caudal vertebrae ; Fig (8) Front view of a caudal vertebra.

a. d., anterior division of the parapophysis ; a. zp. anterior zygapophysis ; a. v. p. anterior ventral process b., bridge between parapophyses ; b 1-2., first and second bridge between the parapophyses cent. centrum ; d. l. g. dorsolateral groove ; g. groove h. a. haemal arch h. sp., haemal spine ; n. pl. neural plate ; n. a., neural arch ; n. sp. neural spine, n. sp. 5., neural spine of fifth vertebra ; p. d., posterior division of the parapophysis ; p. p. parapophysis p. p. 5., parapophysis of fifth vertebra ; p. zp. posterior zygapophysis p. v. p. p. zp. 5., posterior zygapophysis of fifth vertebra p. v. p. posterior ventral process s. p. saccus paravertebralis v. l. g. ventrolateral groove.

B. bagarius (Ham.) Plate-I

1 2

3 4 5

3 cm

8 7 6

<u>B. bagarius</u> (Ham.)

Plate-II

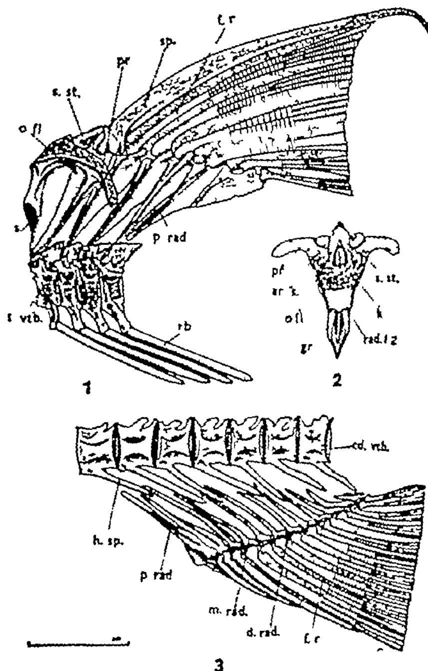
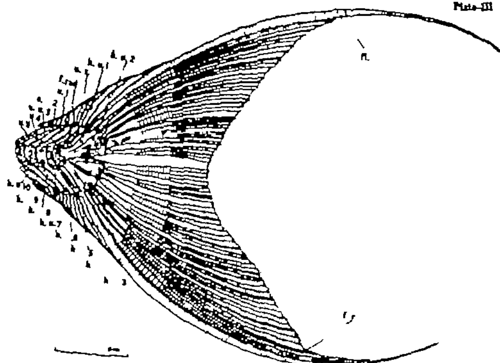


Fig. (1) Dorsal fi... Fig. (2) First ...nd second radials of dorsal f... ; Fig. (3 ... F ... ar. s., articular ...urf...ce for head of pectoral spine ... cd. vtb. caudal vertebra ... d. rad. distal radial ... f ... fin ... ; fl filament ; gr groove ... h. sp. haemal spine ... m. rad. mesial radial ... fl osseous filament ... pr process ... p. rad. proximal radial ... first and second proximal radials fused ... rb rib ... s. st. saddle shaped structure ... s., articular surface for neural spine of fifth vertebra ... sp. spine ... t. vtb. ... vertebra

B. bagarius (Ham.)

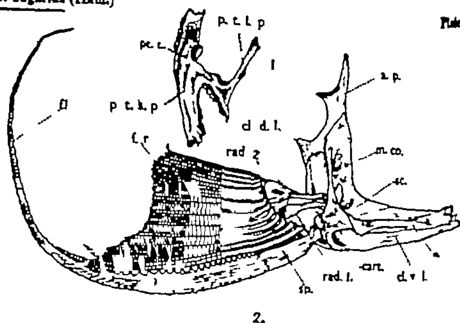
Plate-III



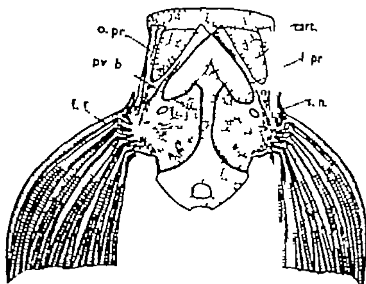
e. 1-5 first, second, third fourth and fifth epurals *fl.*, filament *f. rad*, free radial. *f* fin ray; *h. u. 1 10* first ten hypurals ; *u. s.*, urostyle.

B. bagarius (Ham.)

Plate-II



2.



3



Fig. (1) Posttemporal Fig (2) Pectoral girdle and fi Fig (3) Pelvic g[illegible] fin and [illegible]
a. p., articula process; cart., cartilage; d. d. l. dorsal limb of cleithrum [illegible]
ventral limb of cleithrum [illegible] coracoid; fl filament f. [illegible] fin ray i. pr [illegible]
pelvic bone m. co mesocoracoid; o. pr outer process of pelvic bone [illegible]
pt. t., posttemporal p. t. i. p posttemporal inferior process p. t. s. p posttemporal [illegible]
process rad. 1 2., first and second radials; sc scapula s. n., supraneurory bone [illegible]

THE STRUCTURE AND DEVELOPMENT OF OVULE AND SEED OF PASSIFLORA FOETIDA LINN

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INTRODUCTION

Earlier literature of the Passifloraceae mainly deals with the pre-fertilization development of ovule and it has been summarised by Schnarf (1931) Cook (1909) reported convolutions and growth of pollen tube which persisted inside the embryo sac in *Passiflora adenophylla* In one case he observed the embryo sac completely filled by such convoluted pollen tube Raju (1952 1956) who has studied gametogenesis and seed development of *Passiflora foetida*, *P leschenaultii*, and *P calcarata*, occasionally recorded similar growth of the persistent pollen tube. Padhye and Deshpande (1960) have recently described the male and female gametophytes of *P foetida*.

Netolitzky (1926) has reviewed the development and structure of seed coat and aril of *Adenia venenata*, *Passiflora holosericea* and *P hirsuta*, while Raju (1952, 1956) has given an account of seed coat development in *P foetida*, *P leschenaultii*, and *P calcarata*.

MATERIAL AND METHODS

The material, fixed in formalin-acetic-alcohol, was kindly sent to me by Shri S S. Ramam from Andhra. It was run up through tertiary butyl alcohol series and embedded in paraffin wax. Sections, cut 8 to 20μ thick, were stained with Heidenhain's iron-alum-haematoxylin safranin and fast green or crystal violet and erythrosin Structure of cells of different layers of seed coat was determined in whole mounts prepared by macerating, teasing and tapping the tissue

OBSERVATIONS

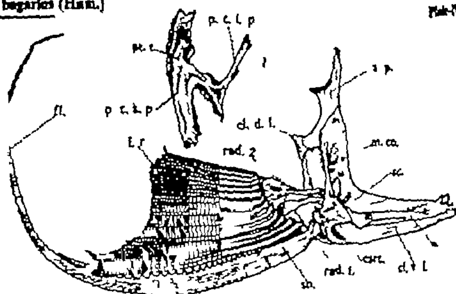
Ovule The ovary raised on an androgynophore, is tricarpellary syncarpous, and unilocular with three parietal placentae. The ovules arise as tiny nascent protuberances in two rows on each placenta (Fig 1) Integument primordia differentiate quite early (Figs. 2, 3) During further development ovules curve and those of one of the placentae come to lie between those of the corresponding lateral placentae (Fig 2) Ultimately they curve through 180° and all ovules in a locule become anatropous (Fig 4) They are crassinucellate and bitegmic. The micropyle is formed of both the integuments and it obliquely faces the ovary wall (Fig 5) This is in contradiction of the recent

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This investigation was completed at the Department of Botany B R. College, Agra.

B. bagarius (Ham.)

Plate-II



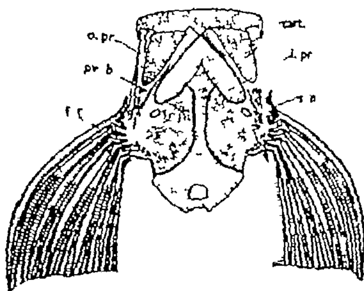


Fig. (1) Posttemporal ; Fig. (2) Pectoral girdle and fin; Fig. (3) Pelvic girdle and fin [illegible] a. p., articular process; cart., cartilage; d. d. l., dorsal limb of [illegible] ventral limb of cleithrum co. coracoid fl., filament [illegible] pelvic bone ; m. co. mesocoracoid; o. pr. outer process of pelvic bone [illegible] pt. t., posttemporal p. t. i. p. posttemporal inferior process [illegible] process rad. 1 [illegible] first and second radials sc., scapula; [illegible]

and some of them remain even multinucleate (Fig 15) In a mature seed the endosperm becomes ruminate and its cells are filled with reserve food material

Embryo In a four-celled proembryo the cells are arranged in three tiers in a T-shaped manner (Fig 16) The terminal tier consists of two juxtaposedly arranged cells, while the basal ones are supperimposed. It appears that the first division of zygote is transverse followed by longitudinal division in terminal and transverse in basal cell The derivatives of the terminal cell divide by transverse wall forming l and l (Fig 17) Cells of l tier undergo vertical divisions at right angles to the previous wall (Fig 18) This is followed by periclinal divisions of these cells giving rise to an outer and an inner group of cells (Fig. 19) The outer layer of cells forms dermatogen which ultimately develops into epidermis. The cells of the inner group form periblem and plerome Simultaneously the constituents of l tier also divide by vertical and transverse walls (Figs. 18 19)

Along with the divisions in derivatives of terminal cell, *ci* divides by an oblique wall (Figs. 17 18) Second division in one of its cells is also by a similar oblique wall forming a group of three cells (Figs. 19 20) Sooner or later *ci* also divides by a transverse wall to form *n* and *n* The *former immediately* undergoes a vertical division (Fig 20)

Subsequently the embryo becomes pear-shaped (Fig 21) and later on, owing to more divisions, in positions corresponding to cotyledons, it becomes heart-shaped (Fig. 22) The cells of cotyledonary primordia divide more rapidly giving rise to two large, thin and straight cotyledons (Fig 23) In mature embryo the vascular supply is procambial in nature. The embryo development of *P foetida* corresponds to the Myosurus variation Onagrad type

Seed Development

Changes in the nucellus and the chalaza After fertilisation the cells of the nucellus increase in size, become vacuolate and are gradually used up by the developing endosperm. The chalazal cup-shaped structure with the vascular elements, becomes more prominent at this stage (Fig 24 *p*) and later on, the cells of nucellus abutting upon this cup-shaped structure become thickwalled (Fig 25)

Changes in the inner integument The inner integument in an unfertilised ovule is three layered. After fertilisation the first visible change is the radial elongation of the cells of its outer epidermis (Fig 26) Later on, these cells are greatly sclerified and possess both simple and ramiform pits on the radial, as well as, tangential walls (Figs. 28, 29) As they are not uniformaly elongated, at places they press on the nucellus and give a somewhat ruminate appearance to the inner surface of the seed coat (Fig 31) These ingrowths, which are confined to the flat surfaces of the seed, lie parallel to its long axis. Trans-longitudinal sections of the seed alone show their presence while in perfectly median longitu

dinal sections no such ruminations are seen (Fig 30) Raju (1956) however figures them in both trans-longitudinal, as well as, median longitudinal sections in *P calcarata.*

The cells of the middle layer increase only a little in size but the inner walls of these cells get thickened in mature seeds (Fig 28) The cells of inner epidermis develop granular contents, take deep red stain with safranin and, along with the other layers of the integument, persist in a mature seed (Fig 28)

Changes in the outer integument Like the inner integument the first visible change in the outer integument is the radial elongation of cells of its inner epidermis. The elongating cells of the micropylar region undergo periclinal divisions which are more pronounced on funicular side and form four or more layers of cells. The radial elongation of cells of inner epidermis of outer integument is also not uniform and against the ruminations these cells are more elongated. They thus help in increasing the ingrowths of the developing seed coat. They remain thin-walled and possess starch grains in the mature seed (Fig 27) Cells of the middle layer are tangentially elongated, while those of the outer epidermis show an increase in size, and in a mature seed, their outer wall is smoothly cuticularised (Fig 27)

Aril Soon after fertilisation the peripheral cells in the vicinity of the hilum start dividing and initiate formation of an aril (Fig 32) Thereafter it grows rapidly and covers the developing seed (Figs 30 31) leaving a small gap at the chalazal end In a longitudinal section aril is found to consist of two to three layers of cells near the distal end but gradually it becomes thicker towards the point of its attachment (Fig 33)

Mature seed The mature seed is enveloped by a succulent aril. On removing this aril, it is found to be flat brownish black structure The outer coat of a mature seed is formed of the three layers of outer integument as described above Its epidermal cells are slightly elongated and smoothly cuticularised The cells of middle layer are tangentially elongated and narrow while those of the inner epidermis are radially elongated and possess starch grains (Figs 27 34)

The inner seed coat is formed by all the layers of inner integument. The outer layer of this seed coat forms the characteristic sclerenchyma sheath (Figs 28 29) and imparts the brownish black colour of the mature seed. The cells of the middle layer show thickening on their inner walls and are persistent This is in confirmation of Netolitzky (1926) who has reported the persistence of the middle layer in *P holosericea* and *P hirsuta.* Raju (1956) has however reported the degeneration and disappearance of this layer in *P calcarata*, *P foetida* and *P leschenaultii* The cells of the inner layer are small and take deep red stain with safranin Similar is the observation of Raju (1956) for the cells of the inner epidermis in *P calcarata*, *P foetida* and *P leschenaultii* while Netolitzky (1926) mentions that it disappears in the mature seeds of *Passiflora* species.

The flat surface of the seed coat on its inner side has ruminations (Figs. 31 35) which do not extend deep into the seed The ruminations of *P foetida* correspond to Spigelia type of Dahlgren (1922) as in this case also the ruminations are formed by the special remnant portion of the seed coat. The formation of similar ruminations in seeds of varied families is also reported by other workers in recent years (Corner 1949 Swamy 1949 Venkata Rao 1953 Raju, 1956)

In a mature seed the nucellus is represented by a few layers of cells only at the micropylar end. On the chalazal end, however it is represented by thick-walled cells along with the cup-shaped structure.

The endosperm in a mature seed is wavy in outline and full of food reserves. The embryo is straight and lies in the middle of the seed. Its vascular supply is of procambial nature.

Summary

The paper deals with the investigation of the structure and development of seed of *Passiflora foetida*. Its ovary is tricarpellary syncarpous and unilocular with parietal placentation. Ovules are anatropous, crassinucellate and bitegmic. Micropyle is formed of both the integuments and it obliquely faces the ovary wall. The vascular supply terminates at the chalazal end of the ovule.

Pollen tube which occasionally convolutes inside the embryo sac is persistent. Endosperm develops normally in embryo sacs in which convoluted parts of pollen tubes are present.

Endosperm development is free nuclear Enucleate cytoplasmic nodules are observed on the micropylar as well as the chalazal side of the free nuclear endosperm. Wall formation is centripetal. Endosperm is persistent and ruminate or wavy in outline in a mature seed Embryo development conforms to the Myosurus variation, Onagrad type.

Both the integuments at the mature embryo sac stage are three layered and take part in the construction of seed coat. Cells of the inner epidermis of the outer integument and the outer epidermis of the inner integument are radially elongated. The latter cells are lignified and possess simple and branched pits on their walls The inner surface of seed coat shows ruminations on the flat surfaces. Mature seed is enveloped by a succulent aril.

I am highly thankful to Professor Bahadur Singh for valuable guidance during the course of this study and also in the preparation of this paper Thanks are also due to D R. K. Singh, Principal, B. R. College, Agra and to Professor C. V Subramanian, Head of the Botany Department, Rajasthan University Jodhpur for facilities and encouragement.

LITERATURE CITED

1 Cook, M. T 1909 Notes on the embryo sac of *Passiflora adenophylla*. *Bull. Torr. bot. Club* 36 : 373-374.

2 Corner E. J H. 1949 : The annonaceous seed and its four integuments. *New Phyt.*, 48 ; 332-364

3. Dahlgren, K. V. O. 1922 : *Die* Embryologie der Loganiaceengattung *Spigelia*. *Svensk Bot. Tidskr.*, 16 : 77-87

4 Netolitzky F 1926 "Anatomie der Angiospermen-Samen Berlin.

5 Padhye M.D & Deshpande B. G 1960 : The male and female gametophytes of *Passiflora foetida* Linn. *Proc. Indian Acad. Sci.*, 52 : 124-130.

6. Raju M. V S. 1952 Embryology of Passifloraceae. *Curr Sci.*, 21 (10) : [illegible]

7 Raju, M V S. 1956 Embryology of the Passifloraceae. I Gametogenesis and [illegible] development of *Passiflora calcarata* Mast. *J Indian bot. Soc.*, 35 : 126-138

8. Schnarf, K. 1931 : "Vergleichende Embryologie der Angiospermen Berlin.

9. Singh B. 1959 Studies in the family Malpighiaceae. I. Morphology of *Thryallis glauca* Kuntze *Hort Advance* 3 : 1 19.

10. Singh, D. 1955 Cytoplasmic nodules in the endosperm of some Cucurbitaceae *Nature* 176 : 607-608.

11 Singh, D. 1959 : Studies on endosperm and development of seeds in Cucurbitaceae and some of its related families. *Ph. D thesis, Agra Univ.*

12 Singh, D. 1960 Studies on endosperm and development of seeds of *Cucumis* [illegible] *L. Hort. Advance* 4 : 89-96

13 Singh D 1962 Cytoplasmic nodules in the endosperm of Angiosperms. *Bull. Torr. bot. cl.* (In Press)

14 Singh, D & Gupta, J S. 1956 Cytoplasmic nodules in the endosperm of *Cucumis sativus* L. *Sci. & Cult.* 22 : 343-344

15. Singh R. P 1959 Structure and development of seeds of Euphorbiaceae *Ph.D thesis Agra University Agra.*

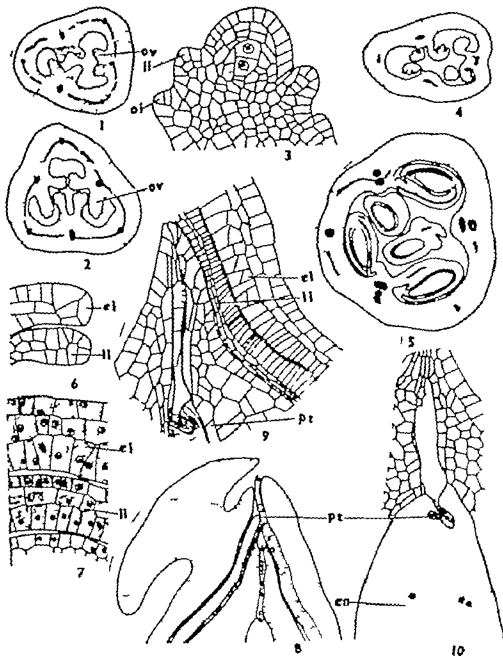
16 Swamy B G L. 1949 : Further contribution to the morphology of the Degeneriaceae *J Arnold Arbor.*, 30 : 10 38.

1 Venkata Rao, C. 1953 : Embryological studies in Palmae *Proc. Indian Sci. Congr.* 42 : 232.

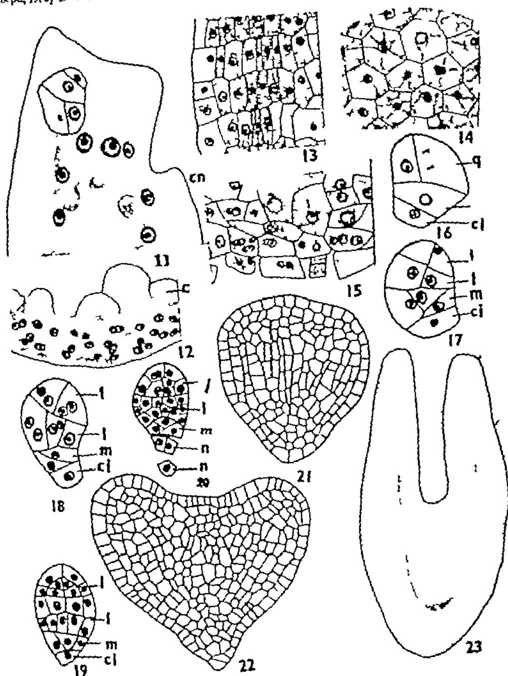
LEGEND

Figs. 1 10 Development of ovule and the pollen tube

Fig 1 T S. ovary showing ovular primordia. X36. Fig 2 same with the [illegible] Fig [illegible] ovules. X36. Fig 3. L. S. part of ovule showing integuments and nucellus. X[illegible] Fig 6 L. S. part of 5 T S. ovary showing further stages in ovule development. X28. Fig [illegible] ovule t an early stage X412. Fig 7 same at mature embryo sac stage X[illegible] Fig [illegible] L. S. micropylar part of an ovule showing pollen tube X105. Figs. 9 10 same [illegible] pollen tube inside the embryo sac. X200. (*en*, endosperm; *ei* exterior integument *o* [illegible] integument *ii* inner integument *ov* ovule; *pt*, pollen tube).


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Figs. 11-23. Endosperm and embryo. Fig. 11 L. S. part of embryo sac, see the enucleate cytoplasmic nodules. X440. Fig. 12. same, chalazal part, X266. Fig. 13 L. S. peripheral part of cellular endosperm with radially arranged cells. X266. Fig 14. Endosperm cells in surface view X266 Fig 15. L. S chalazal part of cellular endosperm. X266. Figs. 16-23. Stages of embryo development (for explanation see text). (Figs. 16-19, X540; Fig 20, X440; Figs. 21 22. X266; and Fig. 23 140) (cn cytoplasmic nodules).

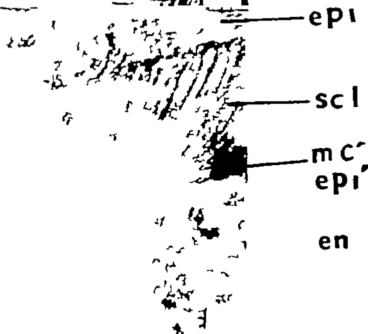

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Figs. 24-33. Stages in seed development. Fig. 24 L. S. chalazal part of young seed X22. Fig 25. same, from mature seed. X22. Fig 26. L. S. part of integument after fertilization. X330 Figs. 27 28. L. S. seed coat parts of mature seed (outer and inner integuments respectively) X200. Fig. 29. T. S. sclereid cells. X200 Fig 30. M. L. S. seed coat [illegible] smooth inner lining of seed coat and transversely cut ruminations in the centre. X[illegible] Fig. 31. T. L. S. seed showing seed coat rumination and the aril. X8.5. Fig. 32. L. S. micropylar part of ovule showing aril primordia. X200. Fig 33. L. S. aril fairly developed seed. X[illegible] (*a*, aril, *ap*, aril primordia; *cp* chalazal pad *ei*, exterior integument or outer integument; *em*, embryo *en*, endosperm *epi* inner epidermis of *ei* *epi*, inner epidermis of *ii*, *epo* outer epidermis of *ei*, *h* hypostase, *ii*, inner integument, *mc*. cells of the middle layer of *ei*, *mc* cells of the middle layer of *ii*; *nu*, nucellus; *scl* sclerenchyma cells of main mechanical layer *vb* vasculla bundle)

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Figs. 34–35. Photomicrographs. Fig. 34 L. s. part of mature seed coat. X280. Fig. 35 same from the region of rumination X280. (en endosperm; epi, inner epidermis of ii; ei inner epidermis of ii; me cells of the middle layer of ii; me' cells of the middle layer of [illegible]; scl sclerenchyma cells of the main mechanical layer)

epo
mc
ep i
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Figs. 34 35. Photomicrographs. Fig. 34 L. s. part of mature seed coat. X[illegible] [illegible] same from the region of rumination. X280 (*en* endosperm *iep*, inner epiderm[illegible] *el* inner epidermis of *ii* *mc* cells of the middle layer of *ii* *mc* cells of the middle [illegible] *scl* sclerenchyma cells of the main mechanical layer)

GRASSES AS COLLATERAL HOSTS OF RUSTS I SUSCEPTIBILITY OF CERTAIN GRASSES TO *PUCCINIA PENNISETI* ZIMM.

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Introduction

Grasses are known to be collateral hosts of cereal rusts. Some grasses have been observed to be susceptible in nature whereas others shown to be so when artificially inoculated with rusts under favourable glass house conditions.

Puccinia penniseti Zimm. the rust of *bajra* (*Pennisetum typhoides* Stapf & Hubb.) appears in fields about the end of September in Uttar Pradesh. *Bajra* is sown with the advent of the monsoon and harvested by late October. The uredo- and teleutospores left over after harvest are subject to intense heat (40-45° C) of the plains during summer. Prasada (1948) reported that the teleutospores of this rust do not require a period of rest for germination and exposure to 45° C for 36 hours kills the spores, although at 35-38° C their viability is not materially affected for 24 hours. Basu-Chaudhary (1955) observed that the high temperature during May and June is hardly suitable for the survival of the uredospores since they lose their viability after a week on exposure to 40-42° C and after two weeks at 30-35 C. Prasada (D Sc. thesis) studied the influence of high temperature on the viability of fresh uredospores and found out that the rust can withstand temperatures upto 100° F (37.8° C) or so without serious injury but 18 hours exposure at 100-110° F (37 8-43° C) impairs the germinability from 80-90% to only traces, while all the spores are killed in 24 hours. At 110-120°F (43-49° C) the spores are killed in 12 hours

Ramkrishnan & Soumini (1948) obtained the aecidial stage of this rust in the laboratory on *Solanum melongena* L. by artificial inoculations. The aecidial stage is found in nature in South India and Poona. So far the aecidial stage of this rust has not been recorded from the plains of Northern India although an aecidial stage on brinjal is reported from Benaras. Besides this rust the aecidial stage of *Puccinia paspalicola* and *Aecidium solani* (Mundkur 1938 and Butler & Bisby 1931) are found on brinjal and it remains to be confirmed which aecidial stage has been collected from Benaras. It is yet to be ascertained if the rust in the aecidial stage can survive the critical months in the plains of Northern India. As stated above grasses are known to be collateral hosts of cereal rusts. Ramakrishnan & Sundaram (1956) reported

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that *Pennisetum orientale* and *P polystachyon* were susceptible to the rust (about 5%) and these grasses may act as collateral hosts. In this investigation many more grasses have been tested for their susceptibility

Method and Material

Seeds of the grasses were obtained from various sources. The grasses were raised in 4 pots (6-8 seedlings) and at 2 leaf stage were inoculated following the usual method of rust infection Pots containing bajra seedlings were kept with each set to serve as control. The leaves were inoculated by applying the inoculum with a lancet needle and the pots were kept in a moist chamber for 48 hours and then transferred to the benches in a glass house The work was carried out in a spore proof glass house and all necessary precautions to avoid contamination were taken. Recording of reaction types was done 8-10 days after the incubation period using the reaction key of Vasudeva *et al* (1953)

Results and Discussion

Twenty grasses were inoculated in the seedling stage and the results are summarized in Table I

TABLE I

Reaction of 20 grasses to *Puccinia penniseti* Zimm.

No	Name	No of trials	No. of seedlings infected/ inoculated	Reaction	Remarks
1	*Cenchrus ciliaris*	3	10/15	R	Reddish brown spots
2	*C setigerus*	3	9/15	R	Yellowish to reddish brown spots in chloratic areas.
3	*Dichanthium annulatum* I. C 2131	2	4/10	R	Chloratic areas.
4	*D caricosum* I W 1029	3	0/15	I	
5	*Heteropogon contortus*	3	4/15	R	Chloratic areas.
6.	*Urochloa mosambicensis*	2	0/10	I	
7	*Pennisetum purpureum*	3	9/15	R	Reddish brown to dark brown spots.
8	*P polystachyon*	1	[illegible]/5	S	Pustules scattered rare rate of type 3.
9	*Panicum maximum*	3	0/15	I	
10.	*Paspalum notatum*	3	0/15	I	
11	*P scrobiculatum*	2	0/10	I	
12	*Brachiaria lata*	2	[illegible]/10	R	Chloratic areas.

(Continued on page 115)

TABLE 1—(Contd.)

Reaction of 20 grasses to *Puccinia penniseti* Zimm.

No	Name	No of trials	No. of seedlings infected/inoculated	Reaction	Remarks
13.	*Chloris gayana*	1	0/5	I	
14	*Sorghum halepense*	1	0/5	I	
15	*Setaria glauca*	2	0/10	I	
16.	*Themeda arundinacea*	2	0/10	I	
17	*Echinochloa colona*	2	0/10	I	
18.	*E. stagnina*	2	0/10	I	
19	Unidentified 1	2	0/10	I	
20.	Unidentified 2	2	0/10	I	

I—Immune R—Flecking or drying of leaf tips Pustules absent if present very small and surrounded by necrotic zones.

S—Pustules big without necrotic zones.

After Vasudeva et al 1953

From the table it will be seen that *Cenchrus ciliaris* *C setigerus* and *Pennisetum purpureum* got weekly infected producing necrotic areas without any pustules while *Dichanthium annulatus*, *Heteropogon contortus* and *Brachiaria* *lata* showed chlorotic zones. The other grasses tested were immune to the rust except *Pennisetum polystachyon* which was susceptible to the rust.

This rust has been recorded on *Pennisetum leonis* from East Africa. Ramakrishnan & Sundaram (1956) inoculated six species of *Pennisetum* viz., *P elephantum* Steud. *P orientale* Rich. *P clandestinum* Hochst. *P hohenackeri* Hochst., *P polystachyon* Sch and *P rupellii* Steud. and two species of *Cenchrus* viz. *C ciliaris* L. and *C setigerus* Vahl. They got slight infection (about 5%) on *Pennisetum polystachyon* and *P orientale*

Pennisetum purpureum reported to be immune to the rust (Ramakrishnan & Sundaram 1956) was found to produce necrotic areas without any pustules. *Cenchrus ciliaris* and *C setigerus* also showed mild infection in contrast to the observations of the authors *Pennisetum polystachyon* was, however susceptible.

SUMMARY

Ramakrishnan & Sundaram (1956) reported that the rust of *bajra* (*Puccinia penniseti* Zimm.) infected *Pennisetum orientale* and *P polystachyon*

and these may act as collateral hosts. The possibility of the recurrence of the disease with the aid of collateral hosts was further explored by inoculating a number of grass species with the rust.

The grasses were raised in 4" pots and at 2 leaf stage were inoculated following the usual method of rust infection. Pots containing bajra seedlings were kept with each set to serve as control. Recording of the reaction type was done 8-10 days after incubation period using the reaction key of Vasudeva *et al* (1953).

It was found that *Pennisetum polystachyon* is susceptible. *Cenchrus ciliaris*, *C setigerus* and *Pennisetum purpureum* got infected producing necrotic areas without any pustules while *Dichanthium annulatus*, *Heteropogon contortus* and *Brachiaria lata* showed chloratic regions only. The other 13 grasses tested were immune to the rust.

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REFERENCES

1. Basu-Chaudhary K. C. 1955 : Effect of temperature on the survival of the Rust of Bajra caused by *Puccinia penniseti* Zimm. *Agra Univ. Jour. Res.* (Sc.) 4 : 31-34.
2. Butler E. J & Bisby G R. 1931 : *Fungi of India. Sci. Monogr. Coun. Agric. Res. India* 1.
3. Mundkur B. B. 1938 : *Fungi of India Suppl. I Sci. Monogr. Coun. Agric. Res. India* 12 : 20.
4. Prasada R. : Morphological and Physiological studies in Rusts. Thesis for D. Sc. degree of the Agra University
5. Prasada, R. 1948 : The Formation and Germination of Teliospores of Rusts. *Indian Phytopath.* 1 : 119-125.
6. Ramakrishnan T. S. & Soumini, C. K. 1948 : Studies on Cereal Rusts I. *Puccinia penniseti* Zimm. and its alternate host. *Ibid* 1 : 93-103
7. Ramakrishnan, T. S., Soumini, C. K. & Sundaram, N. V. 1956 : *Further studies on Puccinia penniseti* Zimm. *Proc. Ind. Acad. Sci.* [illegible] : 190-19[illegible].
8. Vasudeva, R. S. Joshi L. M. & Lele V. C. 1953 : Susceptibility of wild grasses to cereal rusts. *Indian Phytopath.* 6 : 39-46.

A TECHNIQUE FOR ESTIMATING CROP LOSS IN THE RUST (*UROMYCES CICERIS-ARIETINI* (GROGN.) JACZ.) OF GRAM (*CICER ARIETINUM* L.)

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INTRODUCTION

Chester (1950) pointed out that "estimates of disease or losses were generally approximate without any measurements and are often not correct" and suggested that "unless a purely random method of sampling is employed, there is a tendency for a plant pathologist's disease loss estimates to be biased by a complex of several factors".

The rust of gram (*Cicer arietinum* L.) caused by *Uromyces Ciceris-arietini* (Grogn.) Jacz. appears rather late in the season and, therefore, doubts have been expressed as to the losses caused by the parasite. Saksena & Prasada (1955) observed that "the extent of damage caused by the rust has not been actually determined but judging from the premature death of infected leaves the loss in out-turn is probably considerable". In order to ascertain the exact position of the loss, random samples from an infected field in the district of Gwalior (in Madhya Pradesh near Agra) were taken according to the "individual method" suggested by Chester (1950) and disease appraisal and loss estimate carried out.

METHOD AND MATERIAL

During *rabi* 1956-57 one hundred plants were selected at random from a mature crop in a cultivator's field near Gwalior where the disease was prevalent and harvested by cutting them at the base. The specimens pressed between old news sheets were carefully brought to the laboratory at Agra College. Observations were recorded on disease intensity growth (shoot dry weight) and yield as well as of seed and pod numbers. Seed-to-pod ratio (by number) and seed-to-shoot ratio (by weight) were calculated.

Since the rust pustules are found mainly on the leaves the intensity of the disease was measured as percentage of infected leaves in total number of leaves on each plant.

Disease intensity was correlated statistically with the above agronomic characters of the infected plants, and where the 'r' values were found significant regression equations were also calculated on the lines suggested by Greaney (1933 a & b).

RESULTS

Observations on disease intensity growth and yield of gram plants are summarized in Table 1.

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TABLE 1

Disease intensity and certain agronomic characters of the host plants

(Mean of 100 values)

Observation	Range	Mean	Standard Error
Mean per plant			
%Disease intensity	22·72—71·42	43 10	±1 1?
Shoot dry matter in gms.	0·9—4 1	2·69	±0 03
Seed Yield in gms.	0 48—3 59	1·93	±0·07
100 seed weight in gms.	4 81—12 33	9 10	±0·14
Pod number	4—35	19·81	±0·62
Seed number	7—38	21·27	±0 4
Seed to-pod ratio	0·57—1 50	0·94	±0 0.
Seed to-shoot ratio	0 85—3 00	1·46	±0·04

The statistical analysis of data indicates a mean disease intensity of 43 10 (±1 12)% as calculated from the percentage infected leaves, although the intensity varied from 22 72 % to 71 42 %, the frequency being the highest between 30 % and 60 %

TABLE 2

Correlation coefficients (r) between disease intensity and yield characters

Observation	'r' value
Shoot-dry matter	−0·507*
Seed yield	−0·678*
100 seed weight	+0·069
Pod number	−0·673*
Seed number	−0 670
Seed-to-pod number	+0·104
Seed to-shoot ratio	+0·273

Significant at 1 % level.

'r' values (Table 2) calculated between disease intensity and shoot dry matter (minus seeds) at harvest seed yield, seed number and also pod number are significant at 1 % level. Further these values exhibit inverse relation. Correlations between disease intensity and seed-to-pod ratio, seed to-shoot ratio and 100 seed weight are not significant showing thereby little effect of the disease on these agronomic characters

TABLE 3

Regression coefficients ('b) and regression equations between disease intensity and yield characters

Observation	'b value	Regression equation
Shoot-dry-matter	−0 037	y− 2 69=−0·037 (x−43 10)
Seed yield	−0·038	y− 1·93=−0·038 (x−43 10)
Pod number	−0·369	y−19·91=−0·369 (x−43 10)
Seed number	−0 442	y−21 27=−0 442 (x−43 10)

Regression co-fficients were calculated where correlations were significant at 1% level and regression equations calculated There is differential influence of increasing disease intensity on the growth of afected plants and the yield as indicated by the regression equations (Table 3) For better comparison, reduction in growth and yield characters have been calculated for each increase of the disease intensity by 10% The results are given in Table 4

TABLE 4

Reduction in growth and yield characters on increase in disease intensity by 10%

Observation	Reduction (per plant)
Shoot dry matter	0 37 gms.
Seed yield	0·38 gms.
Pod number	3·69
Seed number	4 42

The above results indicate that with increase in disease intensity there is loss in shoot dry weight, seed yield seed number per pod and pod number per plant.

DISCUSSION

Several workers have estimated quantitatively the relationship between disease intensity and the consequent loss in yield. Greaney (1933c and 1934) pointed out a loss in yield of about 7% with stem rust of oats and 8·2% with stem rust of wheat for each increase of disease intensity by 10%. Machacek (1943) reported a loss of 3% caused by root rot of wheat and, Gupta (1934) a loss of 8·9% in the expected yield in the stem-gall disease of coriander with 10% increase in disease intensity Afanasiev & Morris (1942) estimated 3.5% loss in yield for every increase in disease intensity by 5% of

seedling disease of sugar-beets. In the present study a loss of 0.38 gms. in yield per plant has been calculated for every increase in disease intensity by 10%. Further the 'r' and 'b' values and the regression equations for disease intensity and loss in shoot dry matter of number of pods formed or the number of seeds set indicate that these characters are also affected adversely by the disease.

The studies on rust diseases indicate that an increase in disease intensity affects the yield of the host plants adversely either due to decrease in number of grains formed (Caldwell et al 1932 and 1934 Johnston & Miller 1933 and 1934 Mains 1927 and 1930 Pal 1936 Rudorf & Job 1934) or due to defective maturity resulting in shrivelling of the grains (Barclay 1892 Brizgalova 1935 Lukyanenko 1934 Mehta 1950 Neill 1931 Peturson & Newton 1939 Phipps 1938) In the present study loss in yield by the disease is apparently due to the reduction in the number of the seeds formed because 100 seed weight remained uninfluenced.

Summary

With a view to estimate the loss, if any caused by the rust of gram disease appraisal and crop-loss estimates were carried out in a cultivator's field. Correlation coefficients as well as regression values between disease intensity and shoot dry matter yield of seeds 100 seed weight, number of pods number of seeds seed to-pod ratio and seed to-shoot ratio have been calculated on the lines suggested by Greaney (1933 a & b) to investigate the influence of increasing disease intensity of the rust of gram on the above characters.

It was noted that the shoot dry matter seed yield, number of seeds per pod and also number of pods per plant were adversely affected by the disease The remaining agronomic characters, however bore no relationship. A mean loss of 0·38 gms. in yield per plant was found with each increase of 10% disease intensity

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References

1 Afanasiev M. N & Morris, H. E. 1942 : Control of seedling disease of sugar-beets in Montana. *Phytopath.* 32 : 477-486.

2. Barclay A. 1892 Rusts and mildews in India, *Jour Bot.* 30 : 1-8, 40-49

3. Brizgalova, V A. 1935 : (Brown rust of wheat under condition of the [illegible] vendinak zone of the East Siberian District). Trudy po zasch. Rast. [illegible] No. 2 (4) 99-174

4 Caldwell R. M. Kraybill, H R. Sullivan, T T & Crompton, L E. 1932 : The effect of leaf rust *Puccinia triticina* on the composition and yield of wheat in 1931 *Phytopath.* 22 : 5

Original not seen.

5. Caldwell, R. M., Kraybill, H. R.; Sullivan, T T & Crompton, L. E. 1934 : Effect of leaf rust (*Puccinia triticina*) on yield, physical characters and composition of winter wheats. *Jour Agr Res.* 48 : 1049-1071

6. Chester K. S. 1950 : Plant Disease Losses: Their Appraisal and Interpretation. *Plant Disease Reporter Supplement* 193 : 190-362.

7 Greaney F J 1933a : Method of estimating loss in yield from cereal diseases *Phytopath.* 23 : 12.

8. Greaney F J 1933b Method of estimating losses from cereal rusts. Proc. World Grain Exhib. and Conf. Canada 1933, 2 224-236.

9. Greaney F J 1933c Experiments in the Control f oats rust by sulphur dust. *Sc Agr* 13 426-434

10. Greaney F J 1934 : Studies in cereal diseases. XI The prevention of cereal rusts by the use of fungicidal dusts. Canada Dept. of Agric. Bull. 171 N. S. : 90

11 Gupta, J S 1954 : Disease Appraisal of stem-gall of *Coriandrum sativum* L *Indian Phytopath.* 7 53-60

12. Johnston, T & Miller E. C. 1933 : Effect of leaf rust infection on water economy and growth of two wheat varieties. *Phytopath* 23 : 19-20

13. Johnston, T & Miller E. C. 1934 Relation of leaf rust infection to yield, growth and water economy of tw varieties of wheat. *Jour Agr Res.* 49 : 955-981

14. Lakyanenko, P.P 1934 (On the susceptibility of winter wheat hybrids to *Puccinia triticina* Erikss. during 1932 in connection with results obtained in breeding for immunity) No. Cauc. Sel. Cent., Winter wheat seed Lab., Azov-chernomorskoe kraevoe Knigoizdatelstvo, Rostovna-Donu, 1934 46 pp.

15. Machacek, J E. 1943 : An estimate of loss in Manitoba from common rot of wheat. *Sc Agr* 24 70-77

16. Mains, E. B. 1927 The effect of leaf rust *Puccinia triticina* on seed production of Wheat. *Phytopath.* 17 40.

17 Mains, E B. 1930 Effect of leaf rust (*Puccinia triticina* Erikss.) on yield of wheat. *Jour Agri. Res.* 40 417-446.

18. Mehta, K. C. 1950 Control of rust epidemic of wheat in India—A National Emergency *Sci. & Cult.* 15 70-77

19. Neill, J C 1931 : Effect of rusts and mildews on yield and quality of wheat. N Z. *Jour Agr* 43 44-45.

20. Pal, B. P 1936 : Effect of brown rust attack on wheat. *Ind. Jour Agr Sci.* 6 : 127-128.

21. Peturson, B. & Newton, M. 1939 : The effect of leaf rust on yield and quality of Thatcher and Renown wheats in 1938. *Can. Jour Agr Res. C.* 17 : 380-387

22. Phipps, I. F 1938. The effect of leaf rust on yield and baking quality of wheat. *Jour Aust. Inst Agr Sci.* 4 148-151

23. Rudorf, W & Job, M 1934 Untersuchungen bezuglich der Spezialisierung von *Puccinia graminis tritici*, *Puccinia triticina* und *Puccinia glumarum tritici*, sowie ber Resistenz und ihre Vererbung in Verschiedenen Kreuzungen. Ztschr Zucht. A. 19 333-365

24. Saksena H. K. & Prasada. R. 1955 Studies in Gram Rust, *Uromyces ciceris-arietini* (Grogn.) Jacz *Indian Phytopath.* 8 94-98.

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A TECHNIQUE FOR APPRAISAL OF POWDERY MILDEW OF CORIANDER

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Powdery mildews are obligate parasitic ascomycetous fungi growing chiefly on the foliage of angiosperms and cause damage on a wide variety of crops. Reduction in yield resulting from these pathogens has been recorded in barley by 42% (Jensen 1951) hops by 61 71% (Blodgett, 1913 Masters 1855) grapes by 33-90% (Arnaud and Madeline, 1931) peaches by 80% (Fikry 1937) clover by 40% (Horsfall 1930) cucumbers by 75% (Strumbel, 1930) and gooseberry almost cent per cent (Murphy 1930) From time to time several workers like Salmon (1900) Kliko (1922) Jorstad (1925) Skoric (1926) Sawada (1927) Jaczewski (1927) Brundza (1933) Blumer (1933) and Homma (1937) have published comprehensive monographs on this group of fungi. Recently Yarwood (1957) has reviewed the entire literature pertaining to all the members of this family with special reference to morphology taxonomy symptomatology dissemination diurnal cycle, biological specialization, epidemiology and control.

The powdery mildew of coriander caused by *Erysiphe polygoni* D C. appears in Gwalior and other localities near Agra towards the end of March and spreads quickly by the first week of April The characteristic symptoms of the disease are visible on the surface of the leaves and gradually extend to stems and peduncles. The normal development of flowers and fruits is checked and they remain diminutive Young fruits which mature before the appearance of the disease apparently escape loss though get covered by the white powdery mass.

As the disease is more common towards the end of March, the relatively late sown crops are severely affected Although disease causes damage to the crop but no quantitative estimation of the disease intensity and crop losses has yet been done. The present work deals with correlation between disease intensity with loss in yield

METHODS AND MATERIALS

During rabi 1957 58 observations were recorded in mature plants of a late sown crop on disease intensity growth (shoot-dry weight) height number of umbels and yield in a cultivator s field near Gwalior (Madhya Pradesh) where the disease is generally prevalent. Disease intensity was estimated

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in the fields on hundred randomized plants after which they were brought to the laboratory at Agra College for further observations.

Disease Appraisal The visual symptoms were reduced to a quantitative index. The whole plant with a total score of 100 points was divided into three parts main stem carrying 20 points, leaves and umbels with [illegible] points each. The scoring for stem depended roughly on the area of infection while in leaves and umbels on the percentage number affected and the extent of infection in each leaf or umbel

Loss Estimate Chester (1950 p. 295) suggests the 'individual method' for estimating loss by working with hundred pairs of healthy and diseased specimens from the same field This was not possible in the present investigation since the selected field was extensively infested. Thus loss has been estimated as percentage on the total expected yield for each plant as

thus $\text{Loss\%} \frac{a-y}{100} \times 100$ where a is total expected yield i.e. total no of umbels (both healthy and diseased) x mean yield of a healthy umbel and y the actual yield of healthy fruits by the plant.

RESULTS AND CONCLUSIONS

Observations on hundred randomized plants are summarized below

TABLE 1

Observations on disease intensity growth, number of umbels and yield.

Nature of observation	Mean of 100 plants
1 Disease intensity score	11·61 ± 0.33
(i) On stem	35.70 ± 0.35
(ii) On leaves	35.78 ± 0.33
(iii) On umbels	83.06 ± 0.99
(iv) Total	23.09 ± 0.43
2 Height of the plant in inches	
3 Growth (dry weight) of the shoot	1.39 ± 0.09
(without fruits) in grams	20.06 ± 1.17
4 Total number of umbels per plant	0.021 ± 0.003
5 Yield of healthy umbel	00.53 ± 1.13
6 Percentage loss in expected yield	

The mean total disease intensity per plant was 83 [illegible] out of which 11·6% was on stems, 35.7% on leaves and 35.7 on umbels, indicating [illegible] the disease intensity was fairly high on leaves and umbels [illegible] the stem. The yield per plant was very poor although 20.03 [illegible] formed The percentage loss in expected yield amounted [illegible]

Correlation coefficients were calculated between (i) various growth characters and total disease intensity (Table 2) and growth characters and disease intensity on different plant parts (Table 3)

TABLE 2

Correlation coefficients (r) between total disease intensity and growth characters

Shoot-dry weight	−0 115
Height of the plants	−0 159
Total number of umbels	−0.556
Percentage loss in expected yield	+0 780*

Significant t 1% level.

A positive correlation between the total disease intensity and percentage loss in yield was noted while correlation between the total disease intensity and total number of umbels formed was negative. The r values between the total disease intensity and shoot-dry weight and between height and total disease intensity were negative but statistically non-significant, indicating that the total disease intensity has no influence on the growth of the plants which is further supported by the fact that the disease in the appraised crop appeared after the onset of the flowering Since 'r' values were highly significant, regression values (b) were also calculated. An increase of 10% in total disease intensity would result in 10% loss in expected yield and the same would cause a reduction of 5 6 number of umbels per plant.

TABLE 3

Correlation coefficients (r) between disease intensity on various plant parts and certain growth characters

(i)	Disease intensity on stem and total number of umbels	−0·396†
(ii)	Disease intensity on stem and % loss in expected yield	+0·515†
(iii)	Disease intensity on leaves and total number of umbels	−0 443†
(iv)	Disease intensity on leaves and % loss in expected yield	+0·195
(v)	Disease intensity on umbel and total number of umbels	−0·255
(vi)	Disease intensity on umbel and % loss in expected yield	+0·946†

† Significant at 1% level
Significant t 5% level

The disease intensity on stem, leaves and umbels were separately correlated with total number of umbels and percentage loss in expected yield. Positive correlation between loss and in expected yield and disease

intensity either on stem, leaves or on umbels were noted However negative correlations were obtained between total number of umbels and disease intensity Generally a close relationship is observed between disease intensity and loss in yield as observed by Sallans (1935) and Gupta (1954). In the present studies the association between disease intensity and loss in expected yield was also very close and significant The loss in expected yield in a br- sown coriander crop attacked by *Erysiphe poligoni* amounts to 90·5% with a mean total disease intensity of 83%

Regression values (b) for loss in yield and disease intensity have been calculated by several workers in order to forecast losses. Afanasiev and Morris (1942) calculated a loss of 3·5% in a potential crop of (sugar beet) with every increase of 5% disease. Machacek (1943) observed that every 10% increase in root-rot of wheat leads to 3% crop loss. In the present studies a 10% increase in total disease intensity resulted in a 10% loss in expected yield.

SUMMARY

The powdery mildew of coriander caused by *Erysiphe poligoni* D. C. appears towards the end of March round about Agra and Gwalior and spreads rapidly by the first week of April. 100 randomized affected mature plants were selected in a field near Gwalior (Madhya Pradesh) and observations were recorded on disease intensity height of the plants, shoot-dry-weight, grain-yield and number of umbels. A suitable technique for the appraisal of the disease is described.

Correlation coefficients (r) between (I) total disease intensity and growth characters and (II) disease intensities on various plant parts and certain growth characters were calculated wherever 'r' values were significant regression coefficients (b) were also estimated The loss in expected yield per plant amounted to 90·5% with a mean disease intensity of 83%. A positive correlation (+0 788) significant at 1% level was noted between total disease intensity and loss in expected yield. Regression value (b) indicated that every 10% increase in disease intensity resulted in an increase of loss in expected yield by 10° and a decrease in number by 5·6.

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REFERENCES

1 Afanasiev M. M. and H. E. Morris, 1942 Control of seedling disease of sugar beet in Montana. *Phytopathology* 32 : 477 106.

2. Arnaud, G. and Madeleine Arnauld. 1931: Traite de pathologie vegetale. Vol. I. 993
3. Blodgett, F. M. 1913 : Hop mildew Cornell Univ. *Agr. Expt. Sta. Bul.* 328: 281-310
4. Blumer, S. 1933 Die Erysiphaceen Mitteleuropas : 483
5. Brundza, K. 1933: Beitrage Zur Kenntnis der Erysiphaceen. Litauens. Jahrb. Landwirtschaft. Akad. Litauen : 108-197
6. Chester K. S. 1950. Plant disease losses. Their appraisal and interpretation. *Plant Disease Reporter Supplement* 193 190-362.
7. Fikry A. 1937 The control of peach mildew Egypt. *Min. Agr. Tech. Sci. Bul.* 183: 14
8. Gupta, J. S. 1954: Disease appraisal of stem-gall of *Coriandrum sativum* L. *Indian Phytopath.* 7: 53-60.
9. Homma Yashu 1939: Erysiphaceae of Japan, *Jour. Fac. Agr. Hokkaido Univ.* 38 183-461
10. Horsfall, J. G. 1930 A study of meadow crop disease in New York Cornell Univ. *Agr. Exp. Sta. Mem.* 130 139.
11. Jaczewski, A. A. 1927 Powdery mildew fungi: 626.
12. Jensen, N. F. 1951 Powdery mildew of barley Cornell Univ. *Agr. Exp. Sta. Mem.* 305: 89
13. Jorstad, I. 1925 The Erysiphaceae of Norway 116 p. Schriften Utgitt av det Norske Videnskaps Akademi I Oslo I Maten Naturvid
14. Klika, J. 1922 Einige Bemerkungen uber die Biologie des Mehltaus Ann. Mycol. 20 74-80.
15. Machacek J. E. 1943: An estimate of loss in Manitoba from common root-rot of wheat, *Sc. Agr.* 24: 70-77
16. Masters, W. 1855 Result of applying sulphur to hops during the period of their growth. Gard Chron 15 742 743
17. Murphy P. A. 1930 Experiments on the control of the American gooseberry mildew *Jour. Dept. Agr. Ireland* 29: 188-204
18. Salmon, E. S. 1900: A monograph of the Erysiphaceae. Torrey *Bot. Club. Mem.* 9: 292
19. Sallans, B. J. 1933 Study of root rot problem of wheat and barley caused by *Helminthosporium sativum* in Saskatchewan. Canada Dept. Agr Progress Report Dom. Dot. for 1931-34 24-25.
20. Skoric V. 1926 Erysiphaceae croatiae. Prilog fitopatoloskoshistematskoj monografiji mealh pepenica. Annales pro experimentis Forestica Zagreb. I 52 117
21. Sawada, K. 1927 On the systematic investigation of *Erysiphe* in Formosa. Formosa Deptt. *Agr. Govt. Res. Inst. Rep.* 24 55
22. Swaabel, S. J. 1930 Control of powdery mildew of cucumbers by means of disodium hydrogen ortho-arsenate. Comment. Inst. Astrachanensis ad. difensionem Plantarhum 2 21 31
23. Yarwood, C. E. 1957 Powdery mildews, *Bot. Rev.* XXIII, 4 235-302.

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THE CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF THE PASTURE GRASS-BLUE PANIC (PANICUM ANTIDOTALE RETZ.)

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It needs no emphasis that good forage is the foundation of economic and efficient livestock production The hide bound stray cattle on the street are a proof for the extreme negligence towards our cattle wealth. The improvement in the cattle wealth has to be brought about by proper breeding, feeding and healing This article deals only with the feeding aspect. It is an admitted fact that the fodder produced in our country is mostly poor in quality and far below the normal requirement in quantity This quantitative insufficiency and the low nutritive value of the staple fodders are recognized as the chief causes of the cattle deterioration. A liberal supply of nutritious fodders will naturally make up the deficiency both qualitative and quantitative. To achieve this object what we need is the comprehensive knowledge of the various aspects of the fodder grasses.

The studies on the agronomical aspects are in progress at I A. R. I and many soil conservation farms. In U P., at the soil conservation farm, Rehaman-Khera (Lucknow) among the numerous grasses, blue panic is one which is being propagated on a large scale and agronomical studies are being made. Khan (1955) reported that blue panic is a very promising grass with high yielding capacity and deserves wider trial. A similar report regarding the chemical composition was given by Chopra (1956) but nothing is known about its nutritive value and other characteristics Hence a detailed study on its chemical composition, yield of nutrients and nutritive value was taken up

EXPERIMENTAL

The grass was propagated by seeds which were broadcasted at the rate of 6 lb per acre. The field was well prepared with usual manuring The entire study on this grass was planned as detailed below —

1 Study on the chemical composition.

2. Study on the nutritive value.

The first part of the study was confined on the following points —

(a) The chemical composition of the fodder at different stages of growth.

(b) Distribution of the nutrients in different parts lant.

(c) The chemical composition of the plant as influenced by frequency of cutting

(d) The chemical composition and the yield of nutrients per acre when the grass was subjected to the following two treatments —

(i) When cut at monthly interval during rainy season, the first cut having been taken on the 15th July

(ii) When left as such and harvested only once at the end of rainy season

In the second part, it was envisaged to study the nutritive value of the green grass at the stages mentioned below :—

(a) Pre-flowering stage (Plate I)—For a study at this stage four similar adult rams were the experimental subjects

(b) Maturity stage when numerious seed-heads have appeared (Plate II)—At this stage four adult Hariana heifers were employed for the purpose instead of rams.

The metabolic trial was divided into a 20-day preliminary and a 10-day collection period as recommended by Staple (1931) The faeces and urine were collected by the conventional method. The proximate principles were estimated according to the procedure laid down by A. O A. C. (1930 ed) The determination of calcium and phosphorus was done by the modified method described by Talapatra, Ray and Sen (1940)

Results and Discussion

I Studies on the Chemical Composition of Blue Panic Grass

(A) Chemical composition at different stages of its growth —

The chemical constituents estimated in the fodder from very leafy stage to seed-shed stage have been recorded in table 1

It may be noted (Table 1) that the grass is not only rich in crude protein but also in phosphorus in which many of the protein rich-fodders particularly legumes are deficient. Although chemically it is rich at very leafy and leafy stages, keeping in view the height of the plant, maximum return of nutrients can only be expected at the pre flowering stage. The experiments conducted by Das Gupta (1940 and 1942) and Anwarullah (1939 40 and 41) with different legumes and grasses support the findings It will be seen from the data that protein falls markedly with age and with it there is also a decrease in ash which is reflected in the individual mineral constituents with the exception of phosphorus The ether extract also diminishes but at maturity There is a marked increase in the carbohydrate fractions both the N-free extract and crude fibre rising with the onset of maturity The phosphorus and other

extract are high at maturity stage which may be attributed to the seeds which are usually rich in these two substances (Talapatra 1957) The author s findings are in agreement with those of Watson (1951) Lander (1942) and Sen (1938)

At pre-flowering stage, it may be compared with the leguminous fodders like cow pea (Vigna catjung) Russian giant lobia (Vigna sinensis) and berseem (Trifolium alexandrium L.) It is poorer in calcium but richer in phosphorus than the above leguminous fodders.

TABLE 1

The chemical composition of blue panic grass at different stages of its growth (per cent on dry matter basis)

Height and constituents	Growth stages				
	Very leafy	Leafy	Pre flowering	Maturity (in seed)	Seed-shed
A Height in inches	4 20	10 30	24 50	36 70	40 00
Crude Protein	24 87	20 81	17 77	8 95	4 78
Ether extract	2 52	1 98	1 81	2 02	1 74
Crude fibre	15 85	17 95	21 99	25 89	30 72
N-free extract	40 32	43 97	43 36	51 99	52 36
Total carbohydrates	56 17	61 92	65 35	77 88	83 08
Total Ash	16 44	15 29	15 07	11 15	10 40
Calcium (Ca)	0 88	0 71	0 69	0 51	0 46
Phosphorus (P)	0 58	0 48	0 42	0 51	0 21

(B) Distribution of nutrients in different parts of the plant —

The results of Fagan quoted by Watson (*Loc cit*) showed without doubt that the most important single feature in determining the value of a plant is the

relative leafiness at the time it is used as fodder In all measurements of feeding value of the green forage crops and particularly with grass-land herbage, the leaf is the dominating factor With this aim in view the ratio of stem to leaf and the distribution of nutrients in leaf, stem and the whole plant at pre-flowering stage have been studied. The relevant figures are recorded in table 2.

TABLE 2

The chemical composition of the leaf stem and the whole plant at the pre-flowering stage (per cent on dry basis)

Constituents	Whole plant	Stem	Leaf
Crude protein	17 77	12 01	21 60
Ether extract	1 81	1 56	33
Crude fibre	21 99	23 56	20 02
N free extract	43 36	47 37	40 06
Total carbohydrates	65 35	70 93	60 61
Total ash	15 07	15 50	15 34
Calcium (Ca)	0 69	0 49	0 3
Phosphorus (P)	0 42	0 90	0 33

The figures exhibiting the chemical composition of leaf and stem (Ta 2) indicate the normal trend the leaf being richer and stem p[illegible] than the whole plant (Talapatra and Watson *Loc cit*) The stem and leaf r[illegible] of the plant has been found to be 1 1 09 which is also attractive

(C) Chemical composition of the plant as influenced by frequency of c[illegible]

The grass was cut at two three and four weekly intervals The [illegible] cut for each treatment was started in the month of July and the [illegible] cuts were continued till the end of October after which the grass [illegible] the dormant condition throughout the winter without any [illegible] The average chemical composition of the plant for each treatment is recorded in table 3

TABLE 3

The average chemical composition of blue panic grass cut at frequent intervals (per cent on dry basis)

Constituents	Weekly interval		
	2	3	4
Crude protein	15 16	13 10	12 61
Ether extract	3 55	2 92	3 35
Crude fibre	17 65	20 11	20 18
N-free extract	48 40	49 86	49 45
Total carbohydrates	66 05	69 97	69 63
Total ash	15 24	14 01	14 41
Calcium (Ca)	0 53	0 44	0 47
Phosphorus (P)	0 71	0 67	0 73

It is clear from the data (Table 3) that there is not much difference in the chemical composition of the grass at three and four weekly cuts but the yield of fodder was judged more in the latter case. The total outturn of the nutrients when summed up from all the cuttings (from July to October) would, therefore, be higher in this case.

(D) The chemical composition and the yield of nutrients per acre in the monthly cut and once harvested grass —

The chemical composition of the grass has been recorded in table 4

It is evident from the figures (Table 4) that the protein content of the grass at different cuts is higher than when harvested only once at the end of rainy season. In the first fortnight of August, 1957 there was scanty rain which checked the luxuriant growth of the fodder resulting in low protein and higher fibre contents for the cut taken during that month. Phosphorus is high at the maturity stage when no cut was taken

On the basis of the data regarding the chemical composition and the yield of the fodder the total return of certain nutrients per acre in both the cases has been calculated and recorded in table 5

TABLE 4

The chemical composition of the blue panic grass cut at monthly interval as compared to that harvested once at the end of the rainy season (per cent on dry basis)

Constituents	Dates of cutting 15th July	15th Aug	17th Sept.	16th Oct	When harvested only once on 16th Oct.
Crude Protein	18 61	13 81	11 37	11 65	9 [illegible]
Ether extract	1 54	1 38	1 52	2 13	2 11
Crude fibre	17 33	25 82	22 12	21 23	[illegible]
N-free extract	54 01	46 75	53 55	52 31	47 [illegible]
Total carbohydrates	71 34	72 58	75 67	73 51	76 [illegible]
Total ash	16 51	12 30	11 41	12 68	11 [illegible]
Calcium (Ca)	0 64	0 52	0 46	0 57	0 43
Phosphorus (P)	0 50	0 59	0 54	0 54	0 [illegible]

TABLE 5

The relative yield of the fodder and certain nutrients per acre (lb)

Nature of Sample	Green fodder	Dry matter	Crude protein	Calcium (Ca)	Phosphorus (P)
When monthly cut	10 460 00	2 [illegible]98 64	306 [illegible]	11 [illegible]	[illegible]
When only once harvested	8,368 18	2,011 54	185 91	[illegible]	11 [illegible]

It is seen (Table 5) that when monthly cuts during rainy season [illegible] taken instead of leaving the grass to be harvested only once [illegible] half times more crude protein and calcium were available without [illegible] expenditure The author's findings, except for phosphorus are [illegible] with those of Bal 1935) and Lander (*Loc cit*) on different grasses [illegible] no appreciable difference in the yield of phosphorus the probable cause [illegible] to be the formation of seed at the maturity stage

2 The Nutritive Value of the Fodder

(A) Metabolic study at pre-flowering stage —

The composite sample of the fodder with which the digestibility trial was conducted contained 16 52, 1 75, 21.50, 45 42, 66 92 14.81 0 61 and 0.49 per cent. of crude protein, ether extract crude fibre nitrogen-free extract, total carbohydrates, total ash, calcium (Ca) and phosphorus (P) respectively

The palatability of the blue panic fodder as determined by the dry matter consumption is shown below —

TABLE 6

Dry matter consumption by sheep

Animal Number	Body weight (kg)	Dry matter consumption per day (kg)	Dry matter consumption per 100 kg body weight (kg)	Average (kg)
1	40	1 2	3 0	3 13
2	41	1 3	3 2	
3	47	1 6	3 4	
4	42	1 2	2 9	

It may be observed that sheep relished the stuff very much On an average they consumed about 3 kg of dry matter per 100 kg body weight which may be considered very satisfactory Growing calves have also been found to consume the fodder at the rate of 2.8 kg dry matter per 100 kg body weight It appears from these figures that the fodder at this stage is highly palatable to sheep and calves both.

The digestibility coefficients of the various organic nutrients have been detailed in table 7

TABLE 7

Digestibility coefficients of the organic nutrients (per cent)

Constituents	Animal numbers				Average	
	1	2	3	4		
Dry matter	60.25	62 18	64.02	59.56	61.50	± 1.01
Crude protein	70.85	71.88	73 92	71.20	71.96	± 0.69
Ether extract	40.50	41.02	40 27	39 15	40.24	± 0 40
Crude fibre	56.12	57 89	59 42	55.35	57.20	± 0.90
N free extract	69 84	72 17	72 93	68.25	70 80	± 1.08
Total carbohydrates	64 11	66.03	67.90	65.29	65.83	± 0.80

It may be observed from the data (Table 7) that the crude protein digestibility is high and can be compared with that of the legumes such as gur velvet beans and various other clovers (Lander and Dharmani, 1936). As usual with all green feeds, the ether extract digestibility is low. The digestibility of the carbohydrate fractions appears to be normal.

The balances of calcium (Ca) phosphorus (P) and nitrogen have been tabulated below —

TABLE 8

Calcium Phosphorus and Nitrogen balances.

Animal number	Calcium (gm.)	Phosphorus (gm.)	Nitrogen (gm.)
1	+0.52	+1.81	+3.00
2	+1.21	+2.27	+4.27
3	+1.08	+2.56	+5.18
4	+0.33	+1.90	+3.35

The data indicate that the animals can very well be maintained on the positive balances of nitrogen and minerals under blue panic feeding at pre-flowering stage

(B) Digestibility study at maturity stage —

On analysis, a ten-day composite sample of the fodder at this stage when which the digestibility trial was conducted was found to contain 9.00 2.11 28.91 47.98 76.92, 11.85 0.43 and 0.66 per cent of crude protein ether extract, crude fibre nitrogen free extract total carbohydrates, total ash, calcium (Ca) and phosphorus (P) respectively

The palatability of the fodder was determined by the dry matter consumption It was noticed that the mature Hariana heifers on an average consumed only 1.2 kg dry matter per 100 kg body weight It clearly indicates that at this stage the grass is not relished by the cattle because it becomes woody harsh saltish and most probably bitter in taste (Ch pra Loc cit) When it was given along with other green or dry roughages, the animals began to consume as usual.

The data of the digestibility coefficients of the various organic nutrients have been recorded in table 9

TABLE 9

Digestibility coefficients of the organic nutrients (per cent)

Constituents	Animal number 1	2	3	4	Average
Dry matter	53.24	51.52	52.22	50.35	51 78 ±0.62
Crude protein	59 48	55.86	52 21	57.97	56 38 ±1.58
Ether extract	43 81	41.23	39 16	32.19	39 10 ±2.49
Crude fibre	60.59	59 65	58.77	50.63	57 41 ±2.15
N-free extract	53.04	50 66	53.82	46.39	50.98 ±1 67
Total carbohydrates	55 77	53.99	55.65	48.43	53.46 ±1 75

It may be seen that the average digestibility coefficients of all the organic nutrients except crude fibre have decreased at this stage when compared to those at pre flowering stage. The low digestibility of crude protein may be due comparatively to a poor protein content of the fodder (Warth, 1932) There is no appreciable difference in the nitrogen-free extract content of the fodder at these two stages, but its digestibility coefficients are 50.98 and 70.80 per cent at maturity and pre-flowering stage respectively This indicates that at maturity the grass becomes poor in such carbohydrates as are readily digestible by the animals

The balances of calcium (Ca) phosphorus (P) and nitrogen (N) have been recorded in table 10

TABLE 10

Calcium, phosphorus and nitrogen balances

Animal number	Calcium (gm.)	Phosphorus (gm.)	Nitrogen (gm)
1	+4.90	+3.82	−2 12
2.	+4.06	+1.32	−1.81
3	+3 00	+5 11	−3 45
4.	+2.66	−0 51	−2.08

It is clear from the figures that virtually minerals are positively utilized but nitrogen assimilation is negative, although the protein content of the fodder is about 9 0 per cent. Situation such as this suggests the ketabolism of body proteins under the dietary conditions of the experiment. One of the principal factors involved in this may be low ingestion of energy during the metabolic period. Calculations leading to T D N intake partially support this hypothesis. The actual intake of these nutrients amounted to 5 7 lb per 1000 lb

body weight as against the normal requirement of 7.5 lb. in case of [illegible]. It is also possible that the D C. P intake is running short of the requirement of this class of animals, which have not yet ceased to grow although the actual intake amounts to 0 6 lb per 1000 lb body weight—the normal maintenance requirement of an adult cattle. As such it is difficult to conclude at this stage whether the supplementation of this grass with energy or protein rich [illegible] would improve the nitrogen balances.

Digestible Nutrients

From the chemical composition and the digestibility data of the grass at both the stages, D C. P., T D N and S. E have been calculated and compared with those of the other common fodders available in the country (Sen, 19[illegible]

Table 11

The digestible nutrients in the green blue panic grass as compared to those of common fodders of the country

Name of the fodders	Per 100 kg dry materials D C. P (kg)	T D N (kg)	S E. (kg)	Per kg dry material Ca (gm.)	P (gm)
Blue panic at pre flowering stage.	11 89	57.82	44 40	6.1	49
Blue panic at maturity stage.	5 13	47.57	30.20	4.3	56
Berseem (Bihar)	12.56	59 68	43 40	16.6	5
Guar (Punjab)	6 63	48.88	30 90	22.8	17
Senji (Punjab)	12 61	64.01	41 70	13.5	18
Velvetbean (Punjab)	10 66	63.38	31 10	—	—
Oat (Punjab)	10.50	66 70	46.60	4.8	35

A consideration of the data in table 11 indicates that on the basis of the digestible organic nutrients, blue panic grass at pre flowering stage can favourably be compared with the good quality fodders such as berseem, senji, velvet bean and oat Even at maturity stage it is as good as leguminous fodder [illegible]. So far as minerals are concerned, it is poorer in calcium but richer in phosphorus than legumes and many non leguminous fodders It yields 12 kg [illegible] (P) as against 4 kg per acre obtained from berseem (Srivastava, 19[illegible] The acute shortage of phosphorus in our country elucidates the importance of [illegible] grass Not only this, but calcium and phosphorus ratio is also [illegible] whereas in leguminous fodders this ratio is as wide as 10 1 which has been found to be unsuitable for both growing and milch cattle (Johri, 19[illegible]

It has been worked out by Das Gupta (1943) that berseem can replace the expensive concentrates from the ration to the extent of 50 to 75 per cent. As the study reveals, this grass may also replace the concentrates to some extent. On fresh basis, the best food value obtained from the blue panic grass is about 3.0 kg D C P and 14 5 kg T D N per 100 kg of green stuff contain ing approximately 25 per cent dry matter at pre flowering stage. Therefore, 10 kg green blue panic should supply 0.3 kg D C. P and 1 45 kg T D N enough for the production of 4.5 kg of milk which is normally supported by 2 kg of concentrate mixture containing 15 % D C. P It means that 2 kg of concentrate mixture as above is equivalent to 10 kg of green blue panic fodder

Summary

Studies were conducted on the chemical composition and the nutritive value of a protein rich pasture grass, blue panic (Panicum antidotale Retz.) It has successfully been cultivated with seeds and rootstocks both Although it is a perennial grass but due to lack of irrigation facilities, studies were con fined only during the rainy season. The effect of the factors like stage of growth, frequency of cutting etc. on the chemical composition of the plant has been studied. The metabolic trials with the grass at the pre-flowering as well as matu-rity stage have been conducted on sheep and cattle as the experimental subjects respectively

The chemical composition of the grass at different stages of its growth indicates that it is rich in protein content till the pre-flowering stage after which it abruptly falls at maturity and seed-shed stages. It is not only a good source of phosphorus (0 42 to 0.58%) but maintains an excellent Ca and P ratio (1.5 1)

Studies on the distribution of nutrients in different parts of the plant show that leaves contain 21 6% and stems 12.01 per cent of crude protein.

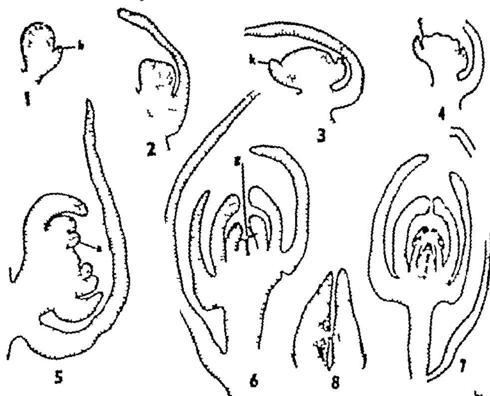
The data on the chemical composition of the fodder when subjected to frequent cuttings reveal that the maximum yield of nutrients may be obtained by cutting at four weekly intervals.

It is evident from the nutrients-yield data that the grass should be cut at the monthly interval instead of harvesting only once at the end of the rainy season. The yield of the crude protein is about 507 and 186 kg., when cut at monthly intervals and once in the end respectively The yield of green fod-der is about 300 mds. per acre.

The fodder is palatable at pre flowering stage to all classes of livestock. Sheep can consume on an average 3 kg of dry matter while young growing calves 2.8 kg per 100 kg body weight. At maturity fodder becomes unpalatable.

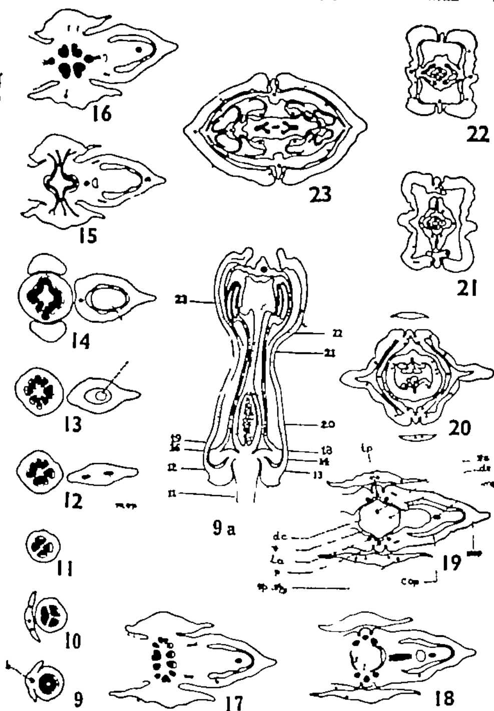
The D C. P T D N and S E. contents of the blue panic grass have been calculated as 11.89 57.82, 44 40 kg at pre flowering and 5 13 47.57 30.20 kg at maturity stage respectively per 100 kg dry stuff. The nutritive value

to form three anthers each, but not before all the floral parts have developed. The nectaries develop late after the microspore mother cells have been formed.



Later on an intercalary growth between the sepals and the bract results in a long pedicel (Figs 6 7) The hypogynous ovary develops due to an growth only of the peripheral tissue of the central mass. As the growth ceases in the centre at an early stage this results in the formation of a tubular structure (Figs 7 8) The ovary cavity shows meristematic activity along two longitudinal lines opposite the sepals organising the placentae which bear ... anatropous ovules Figs 21 25)

External morphology of the flower The pale yellow flowers are borne ... terminal clusters of 8 to 12 flowers Each flower is subtended by a ... bract and bears two antero-posterior sepals The four petals are arranged in two whorls the outer two large saccate to accommodate the nectaries ... almost wholly enclose the inner parts The inner ones are comparatively smaller thin and membranous except at their tips The inner petals are coherent at the tips and fused with the outer ones up to some distance There are two lateral tripartite stamens whose anther is made up of a central ... part flanked on either side by two lateral monothecal members Each ... is continued below with the nectary of its side The gynoecium is ... syncarpous and unilocular with a long and hollow style terminating ... stigma Large number of anatropous ovules are borne on two parietal ... (Figs 21 25) Flowers are highly protandrous. Fruit is an ... capsule ...



Vascular supply of the flower Figure 9a shows the longitudinal course of vascular supply along with the levels bearing the number of the corresponding cross-sections of the flower

Soon after the floral supply leaves the inflorescence axis, the bract trace is given out. To start with it is a single trace (Fig 9) which soon after entering the bract trifurcates (Fig 10) The vascular ring now breaks up into f... endarch collateral bundles (Fig 11) These divide about three times ea... (Figs 12 13) and ultimately fuse laterally (Fig 14) forming a somewhat refor... ring with four ridges and four grooves corresponding to the four sides of the flower

Sepal traces arise from the anterior and posterior ridges of the ring (Fig 15) Each sepal receives three distinct traces but interestingly the third trace from the left divides soon and therefore at the base one observes four bundles (Figs. 16, 17) Higher up, however the other lateral bundle also undergo... division forming in all five bundles (Figs. 18, 19) of these the lateral branches fall short and only the three original bundles supply the apical region of a sepal (Fig 22)

The rest of the ring again splits and from the lateral two bundles arise the traces for the outer petals (Fig 16) This vascular supply dives down int... the spur (Figs 9a, 12 15) and again turns up in its distal part. Each trace gives out two branches on either side (Fig 17) Their further branching higher up results in several commissural veins on either side of the median bundle (Fig 21) It may be noted as would be expected that the median bundle in its downward course in the spur shows inverse orientation (Fig 12, m?)

From the remaining bundles of the centre, two adjacent bundles of the anterior and posterior sides fuse with each other and supply the inner petals (Figs. 17 19 ip) One bundle on either side of the petal trace also travel outside and finally come to lie out of the basal region of the gynoecium (Fig 18) All these bundles continue their upward course for some distance and after reaching the point where the inner petals get feed from the outer ones, the petal trace divides into three (Fig 21) and then enters the free part of the petal (Fig 23)

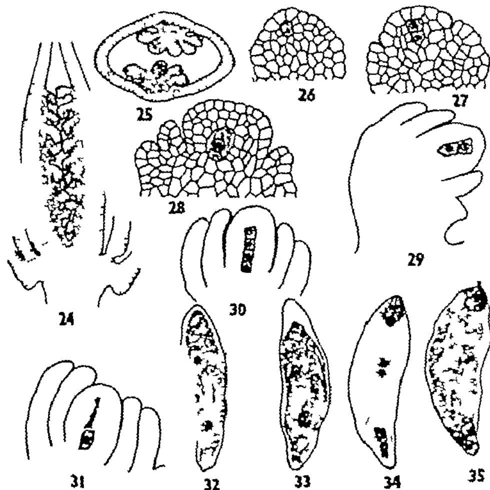
Each stamen receives three traces, the median enters it from the very base (Figs. 16, 17 18 19 ms) while the laterals enter higher up only after ... from their position i.e. on either side of the petal bundle (Figs. 20 21 ...) The median supplies the dithecous anther while the lateral two the monothecous ones (Fig 23) We thus see how the three anthers of one stamen group ... their supplies from three diverse sources.

After the supplies to the sepals, petals and the stamens have been given out, the remaining bundles in the centre further ramify into smaller ... just at the base of the superior ovary (Fig 18) and later on ... opposite the sepals fuse to form the ventral carpellary traces on either ... 19) These travel upward in the region of the two parietal placentae ... the ovules (Fig 20) extending further in the style The small bundles ... the ventral carpellary traces perhaps also fuse as is evident from ...

number and bigger size (Fig 19 *dc*) During their further course they branch, fuse and ultimately the style shows only four bundles i.e. two ventral carpellary and two dorsal carpellary traces (Figs. 21 22) At the base of the stigma the ventral carpellary traces divide and the resulting halves deviate and approach the dorsal carpellary traces, finally fuse with them (Fig 23) Interestingly the resulting two bundles also divide and redivide in the stigmatic lobes their ultimate branches supply the flattened stigma

Ovule The ovular primordia to start with are orthotropous but finally becomes completely anatropous The ovules of *Dicentra scandens* differ in this character from those of *Fumaria* which has campylotropous ovules (Saksena, 1954)

The ovules are bitegmic and the primordia of the inner integument differentiates slightly in advance of the outer one (Fig 27) Initially both the integuments are two cell thick (Fig. 28) The integuments grow vigorously (Figs

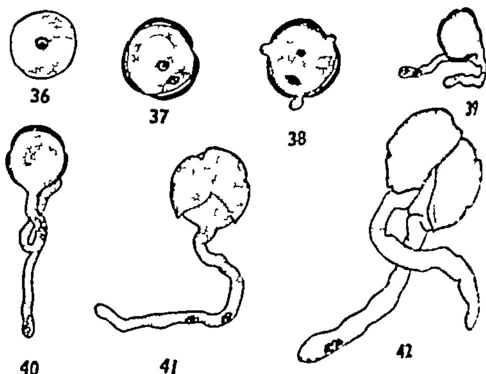


29-31) and soon overtops the nucellus (Fig 46) The micropyle is formed by both the integuments. The ovular supply terminates at the chalaza (Fig 4

Megasporogenesis The single female archesporial cell differentiates in the nascent nucellus (Fig 26) It divides periclinally to form the primary parietal and the megaspore mother cell (Fig 27) The former by periclinal and anticlinal divisions forms the parietal tissue which is usually two cell thick sometimes even three-celled. The megaspore mother cell elongates (Fig 3) and undergoes meiosis to form dyad (Fig 29) and subsequently tetrad (Fig 3 The degeneration of the three micropylar megaspores start soon after the formation and the chalazal one functions (Fig 31)

Megagametogenesis The functional megaspore increases in size and its nucleus divides The two nuclei move to the opposite poles of the embryo sac (Fig 32) which after undergoing division produce four nuclei (Fig 3 All the four nuclei after division give rise to eight nuclei. The organised embryo sac has an egg apparatus at the micropylar end, three antipodals at the chalazal end and the polars lying in the centre of the embryo sac (Fig 3 Thus, the embryo sac development conforms to the Polygonum type

Microgametogenesis The anther wall consists of epidermis, an endothecium whose cells develop fibrous thickening later on single ephemeral middle layer and tapetum which is of secretory type and has multinucleate cells. Microsporogenesis is simultaneous and the cytokinesis takes place by furrow



The young microspore is somewhat spherical with the nucleus in the centre and homogenous cytoplasm alround (Fig 36) A thick exine is soon formed leaving three germ pores. The microspore nucleus after a period of rest divides into two to form a small generative cell and a large vegetative cell (Figs. 37 38) The mature pollen grains are bicelled and in sections appear to be colpate and triporate.

The development of male gametophyte of *Dicentra scandens* exhibits an array of interesting features some of which are new to the Fumariaceae A remarkable point hitherto unreported is the germination of the pollen grains *in situ*. Other points are the germination of the unseparated pollen grains (Fig. 42) and the frequent occurrence of branched pollen tubes (Figs. 39 40)

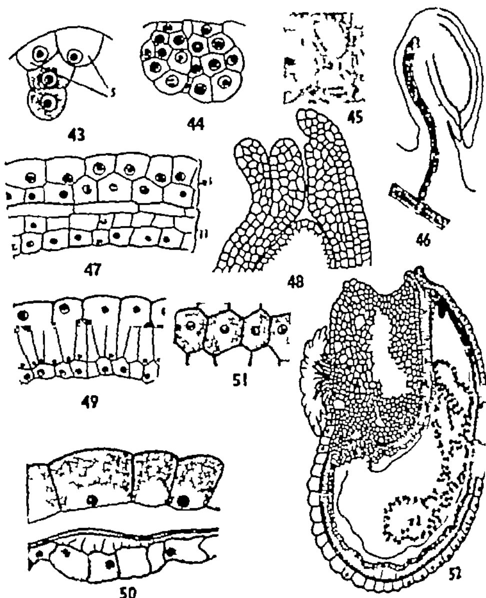
The generative nucleus divides in the pollen tube (Fig 40) and the sperms are clearly made out as dark tiny structures (Figs 39 41 42) In the pollen tube no trace of the vegetative nucleus is seen Saksena (1954) has also reported early disorganisation of the vegetative nucleus in *Fumaria parviflora*.

Pollination and Fertilization The cleistogamous, protandrous nature of the flower the presence of nectary and spur are all indicative of entomophilous pollination. The flattened stigma has four receptive spots and the pollen grains may reach it ungerminated or after germination *in situ*. After pollination the receptive spots are seen to be densely studded with germinated pollen grains with pollen tubes of variable length. The pollen tubes show considerable coiling before gaining entry into the stigma (Figs. 54 55) Later on, they grow along the walls of the hollow style.

The whole mounts of ovaries show the pollen tubes entering the ovule through the micropyle It may or may not injure a synergid while getting into the embryo sac (Fig 35) Persistent synergids (Fig 43) are usually met in earlier stages of seed development as has also been reported by Saksena (1954) in *F parviflora*.

Endosperm and Embryo The endosperm development is nuclear The primary endosperm nucleus divides before the zygote and subsequent divisions are rapid (Fig 32) The nuclei, at this stage, are connected to one another by dense cytoplasmic strands (Fig 45) The wall formation is centripetal and the endosperm cells are polygonal, compact and full of food reserves.

The zygote divides transversely (Fig 43) as in *Fumaria officinalis* (Souèges 1941a, b) *F parviflora* (Saksena, 1954) and *Corydalis lutea* and *C cheilanthifolia* (Souèges, 1946a, b, c) Further details of the embryo development could not be observed but it may be recorded that *Dicentra scandens* occasionally shows the development of a second embryo quite in the position of one of the synergids (Fig. 44) The two embryo in all the observed cases showed an almost equal number of cells. Polyembryony has not been recorded for any other member of the Fumariaceae



Seed development The cells of the nucellus show degeneration from the early stage of the female gametophyte and by the time the embryo sac is organised, it is almost completely absorbed excepting a fragmentary layer on the sides and a few cells thick tissue at the chalazal end. The ovules show the development of a hypostase at the base of the embryo sac. The hypostase develops as an irregular mass of thinwalled cells which are feebly visible in the longitudinal section of an ovule; however, in the whole mount of the ovules prepared after treatment with caustic potash and staining with safranin and aniline blue it appears as a conspicuous structure inside each ovule (Fig. 53).

Both the integuments take part in the construction of the seed coat. As described earlier the outer is two and the inner three cell layer thick (F[illegible]

except at their micropylar ends where the thickness is more (Fig 48) The first apparent change is the disintegration of the outer epidermis of the inner integument, and the radial elongation of the cells of its middle layer (Figs. 49 50) Finally this layer is also disorganised (Fig 50) The inner epidermis of the inner integument also increase in size and after distortion persist in a mature seed (Fig 50)

The inner epidermis of the outer integument also starts disorganising with the outer epidermis of the inner integument. The position of both these layers in a growing seed is marked out by black streaks (Figs. 49 50) Simultaneously the outer epidermis of the outer integument undergoes a remarkable increase in size. Its cells gradually change in colour from dirty white through brown to finally black. They get heavily cutinised bear simple pits on their walls (Fig 51) and form the hard, black and somewhat brittle outer seed coat. Thus in *Dicentra scandens* the seed coat is formed of two distinct covers (Fig 50) an outer testa of black cutinised cells and an inner tegmen of small distorted cells of the inner epidermis of the inner integument. Saksena (1954) also mentions that the seed coat of *Fumaria parviflora* consists of outer epidermis of outer and the inner epidermis of the inner integument.

Discussion

The nature of androecium of the Fumariaceae is controversial. Bernhardi (1833) opined that it consists of 4 dithecal stamens, two lateral and two median Laterals remain as such and medians bifurcate each half getting attached to the two lateral dithecal stamens. This view is also advocated by Lindley (1853) Wydler (1859) and Saunders (1932, 1937) Gray (1880) and Eichler (1875-1878) think that there are only two lateral stamens, each bearing two monothecal members as stipules. Rendle (1925) considers that there are only two tripartite stamens Čelokovsky (1895) Hooker (1872) Duthie (1903) Johnson (1930) and Lawrence (1951) regard that there are six stamens, diadelphous, two lateral dithecal and four median reduced and monothecal. Arber (1931) on the basis of the floral anatomy also concludes that there are six stamens. Norris (1941) believes that the stamens are basically in two whorls and derived from the ancestral forms having eight monothecal stamens, the present dithecals represent the fused four monothecals. Puri (1951) however doubts the validity of this interpretation because of lack of convincing proof.

It also appears difficult to agree with Bernhardi (½+1+½) popular hypothesis because of the independent vascular supply to all anthers The presence of the median petal supply (Fig 19 p) between the supplies of two monothecous anthers (Fig 17 la) from the very base makes it difficult to imagine their connection with each other Arber (1931) also writes for the Fumariaceae in general I doubt that two bundles separated by a third bundle between them can really be halves of the same bundle" Saunders (1937) on the other hand, explains this on the basis of time relation hypothesis. According to her the presence of the median petal supply in anterior-posterior posi

tion at the time when traces for median stamens arise hinder the consolidation of its elements into a single bundle. Further as the traces arise before determinate phase has been reached this condition is due to fractionation of the corresponding member Thus two half stamens develop in the anterior and posterior positions instead one whole stamen The independent vascular supply and monothecous nature of the anterior-posterior stamens is better explained through reduction of dithecous stamens in these positions rather by precocious branching or fractionisation of a single dithecous stamen.

From the study of floral anatomy of *Dicentra scandens* the present authors are led to believe that there are six stamens which are arranged in a diadelphous manner 2 lateral dithecous and 4 median which have undergone reduction. Supposing it be true the question follows as to the cause of reduction of the median stamens. It appears that the monothecous anthers are the result of some physical cause to which the four median anthers have been subjected. In the flower of *Cephalostachyum virgatum* (Gramineae) with monothecal anthers, the reduction has been considered due to development pressure (Gordon, 1864) This supposition it is felt, can be extended on to the Fumariaceae. This pressure hypothesis is clearly supported by the condition of the inner petals A glance at figure 23 shows how the median petals are nipped between the margins of the two lateral petals and how its midrib emerges in the small space between them as if to seek an outlet from its growth-under-pressure Further more the compression in the antero-posterior plane of the flower of *Dicentra*, *Fumaria*, and *Corydalis* may also perhaps be attributed to the pinching effects of the two antero-posterior sepals which though inconspicuous in older stages are relatively well developed in the younger stages (Fig 7)

The investigations on the morphology and floral anatomy of *Dicentra scandens* also enable the authors to deduce some conclusions regarding the systematic position of the genus. The systematists differ in opinion for the circumscription of the Fumariaceae Engler & Prantl (1889) Hallier (1912) Bentham (1915) Engler & Diels (1936) Willis (1936) Fedde (1936) and Goodrich (1950) maintain it as a sub-family of the Papaveraceae while De Candolle (1821)[2] Bernhardi (1833) Lindley (1853) Hutchinson (1926 59) and Lawrence (1951) treat it as a separate family of Rhoedales. Saksena (1954) has recently justified the family status of Fumariaceae On the basis of external and internal morphology of the flower of *Dicentra scandens* it can be concluded that the genus *Dicentra* should be regarded as least specialised among *Dicentra*, *Corydalis* and *Fumaria* It is a generally accepted view that actinomorphy has preceded zygomorphy and similarly ovary with larger number of ovules is more primitive than one with the reduced number In *Dicentra* the flowers are regular as against markedly zygomorphic flowers of *Corydalis* and *Fumaria* and ovary has a very large number of ovules on two parietal placentae in contrast to the few or more ovules of *Corydalis* and only two of *Fumaria*

2 Quoted in Lindley 1853)

Summary

The flowers of *Dicentra scandens* Walp are borne on terminal racemose clusters. The floral parts arise in acropetal succession of two sepals, four petals in two whorls, six stamens forming two groups of three each and a superior bicarpellary and unilocular pistil with parietal placentation.

The flower receives a branch supply from which the first trace to depart is that of the bract. The bract supply trifurcates after entering it. Later on the cylinder breaks up into smaller bundles which fuse to form a stellate structure. At this stage from anterior and posterior positions the vascular supply to the sepals arises. This is followed by the supplies to the outer petals and that of the inner ones in sequence. The three anthers of each stamen group receive their vascular supply from three diverse sources The remaining bundles of the cylinder form the carpellary supply

Ovules are bitegmic, crassinucellate and anatropous and develop a hypostase after fertilisation. Development of embryo sac is of Polygonum type.

Microsporogenesis is simultaneous and cytokinesis is by furrowing Pollen grains are shed at bi-celled stage. They are colpate and triporate. They may germinate in situ and rarely while still in tetrads. Pollen tube is frequently branched. Germinated pollen grains on the stigma show considerable coiling of the pollen tube before entering the stigmatic tissue.

The endosperm development is nuclear and the first division of the zygote is transverse. Polyembryony is also recorded.

Seed coat develops from both the integuments. Initially the outer is two layerd and the inner integument is three cell-layered, except at their tips where the thickness is more. During seed development all the layers disorganise except the outer epidermis of the outer integument and the inner epidermis of the inner one The cells of the former get cutinised, turns black and form testa while those of the latter remain small and thinwalled. The mature seed is albuminous.

On the basis of these studies the nature of the androecium and the systematic position of *Dicentra* in the family is discussed The androecium is shown to consist of six stamens, diadelphous, two dithecous lateral and four median monothecous and reduced Genus *Dicentra* is considered least specialised among *Dicentra*, *Corydalis* and *Fumaria*.

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Literature Cited

1. Arber A. 1931 : Studies in floral morphology III. On the Fumarioideae with special reference to the androecium. *New Phytol.* 30 317 334

2. Bernhardi J J 1833 Ueber den Charakter und die verwandtschaft der Papaveraceen und Fumariaceen *Linnea* 8 401-477
3. Bessey C E 1915 The phylogenetic taxonomy of flowering plants *Ann. Mo. Bot. Gard* 2 : 109-164
4. Celakovsky L. J 1893 Das Reductionsgesetz der Bluthen Sitzungsber. K.b. Ges. Wiss. Math. Nat Cl. 142.
5. Duthie J F 1903 Flora of the upper Gangetic plain Calcutta.
6. Eichler A. W 1875-1878 "Bluthendiagramme Construirt und erlautert. 2 Vols. Leipzig.
7. Engler A. & Diels L. 1936 ; "Syllabus der Pflanzenfamilien. Berlin.
8. Engler A. & Prantl, K. 1889 ; Die naturlichen Pflanzenfamilien. Leipzig
9. Fedde F 1936 ; Papaveraceae unterfamilie III Fumarioideae in Engler & Prantl "Die naturlichen Pflanzenfamilien." Leipzig.
10. Gordon D A 1861 ; Memoire sur les Fumariees a fleurs irregulieres et la cause de leur Irregularit *Ann. d Sci nat. Ser* 5 (Bot.) 2 : 772-256.
11. Gray A. 1880 ; "Structural Botany " New York.
12. Gundersen A. 1930 ; "Families of Dicotyledons Waltham Mass. U S A
13. Hallier H 1912 ; L originale et le systeme phyletique des Angiosperms A ... Ned III (B) 1 : 146-231
14. Hooker J D 1872 ; "Flora of British India. London
15. Hüm G.A. 1906 Beitrage zur Morphologie und physiologie der Antipoden. Bot. Jb. 20 : 77 174
16. Hutchinson, J 1926 1959 "The families of flowering plants 2 vols. London.
17. Johnson A M 1930 "Taxonomy of the flowering plants. New York.
18. Lawrence G H M 1951 ; "Taxonomy of the vascular plants. Macmillan & Co. New York.
19. Lindley J 1853 "The Vegetable Kingdom." Bradbury & Evans London.
20. Norris, T 1941 Torus anatomy and nectary characteristic as phylogenetic criteria in the Rhoedales. *Amer J Bot* 28 : 101 113.
21. Puri V 1951 The role of floral anatomy in the solution of morphological problems *Bot Rev* 17 : 471 553
22. Rendle A B. 1925 ; "The classification of Flowering plants. Vol. II Cambridge
23. Saksena H B 1951 ; Floral morphology and embryology of Fumaria ... *Phytomorphology* 4 109-417
24. Saunders E. R. 1932 ; On some recent contributions and criticisms ... morphology in Angiosperms. New Phytol. 31 : 171 19
25. Saunders, E R 1937 Floral Morphology new outlook ... Cambridge
26. Schnarf K. 1931 Vergleichend Embryologie der Angiospermen. Berlin.
27. Souèges R 1911a Embryogénie des Fumariacees. L origine d ... corps de l'embryon chez le *Fumaria officinalis* L. *C. R. Acad. Sci Par* 213 : 5... 53
28. Souèges, R. 1911b Embryogénie des Fumariacées La différenciation des régions fondamentales du corps chez le *Fumaria* ... *C R Acad. Sci Par* 213 : 699-701
29. Souèges R. 1916a ; Embryogénie les Fumariacées Les premiers termes du développement de l embryon chez le *Corydalis* ... DC. ... 222 161 163.
30. Souèges, R 1916b Embryogénie des Fumariacées. La différenciation des régions fondamentales du corps chez le *Corydalis* ... DC ... 222 : 233 ...5

31. Souèges, R. 1946c Embryogénie des Fumariacées. Developpement de l embryon chez le Corydalis cheilanthifolia Hemsl C. R. Acad. Sci. Paris 222 523-521

32. Willis J C. 1936: "A dictionary of the flowering plants and ferns. Cambridge

33. Wydler H. 1859 : Kleinere Beiträge zu Kenntnis einheimischer Gewächse. Flora 42 : 289-318.

LEGEND

Figs. 1-8. Stages in the organogeny of the flower of *Dicentra scandens* x55. (ndroecium *br* bract; *c*, corolla *g* gynoecium; *k* calyx)

Figs. 9a—23. Vascular supply of the flower of *D. scandens*. For explanation see text. (Fig. 9a, x7; rest all 14) (*b* vascular supply of the bract *cop* commissural vein f outer petals; *dc*, dorsal carpellary trace; *ip*, inner petal trace *la*, vascular supply to lateral anther *ma*, median anther supply mop median bundle of outer petal *n*, nectary *sp*¹—*sp*₂, sepal bundles *vc* entral carpellary trace)

Figs 21-35. Megasporogenesis megagametogenesis nd fertilisation of *D. scandens*. Figs. 24, 25. L.S. and T S ovary espectively 14 Fig 26 L.S. part young ovule showing hypodermal archesporium. x550. Fig 27 L S ovule showing primary parietal and primary sporogenous cell x265 Fig 28 L S ovule with megaspore mother cell capped by parietal layers x265 Fig 29 same, showing dyad x265 Fig 30 same tetrad of megaspores x265 Fig 31 same showing functional megaspore capped by degenerating ones x265 Fig 32-34 stages in embryo sac development x615 Fig 35 L S embryo sac with pollen tube x615

Figs. 36-42 Development of male gametophyte of *D scandens* Fig 36 uninucleate microspore x265 Figs 37 38 Bicelled pollen grains x265 Fig 39 40 Germinated pollen grains showing branched pollen tube x265 Fig 41 Pollen grain with unbranched pollen tube x265 Fig 42 unseparated pollen grains showing germination x265

Figs 43-52 Embryo and seed development of *D scandens* Fig 43 L.S part of the embryo sac showing bicelled embryo with persistent synergids x615 Fig 44 same, showing twin embryos x615 Fig 45 L S part of free nuclear endosperm, note that the uclei are connected by denser cytoplasmic trands x615 Fig 46 L S ovule x55 Fig 47 L S part of integuments befor fertilisation x615 Fig 48 L S micropylar part of an ovule x265 Fig 49 L S part of integument fter fertilisation x615 Fig 50 L S part of seed coat, x265 Fig 51 Cell of seed testa in tangential section, showing pitting x55 Fig 52 L.S growing seed x80 (*s*, synergid *ei*, exterior or outer integument, *ii*, inner integument)

Figs. 53-55 Photomicrographs. Fig 53 Whole mounts of ovule showing hypostase x38. Fig 54 whole mount of stigma, note the germinated pollen grains x65 Fig 55 A part from fig 54 enlarged. 230. (*h*, hypostase)

— Bernhardi J J 1833 Über den Charakter und die verwandtschaft der Papaveraceen und Fumariaceen. *Linnaea* 8 : 401-477

3. Bessey C E 1915 : The phylogenetic taxonomy of flowering plants. *Ann. Mo. Bot. Gard.* 2 : 109-164

4 Celakovsky L J 1895 : Das Reductionsgesetz der Blüthen Sitzungsber. Kön. Ges. II kt. Af th- Nat. Cl. 142.

5. D thie J F 1903 "Flora of the upper Gangetic plain Calc tta.

6. Eichler A. W 1875-1878 Blüthendiagramme Construirt und erläutert. 2 Vols. Leipzig

7 Engler A. & Diels L. 1936 "Syllabus der Pflanzenfamilien. Berlin.

8. Engler A. & Prantl, K. 1889 "Die naturlichen Pflanzenfamilien. Leipzig

9 Fedd F 1936 : Papaveraceae unterfamilie III. Fumarioideae in Engler & Prantl "Die naturlichen Pflanzenfamilien." Leipzig

10. Gordon D A 1864 : Memoire sur les Fumariees a fleurs irregulieres et sur la cause de leur irregularite *Ann. d Sci nat. Ser* 5 (Bot.) 2 : 72-239

11 Gray A. 1880 : "Structural Botany" New York.

12 Gundersen A. 1950 : "Families of Dicotyledons" Waltham Mass. U S A

13 Hallier H 1912 : L origine t le systeme phyletique des Angiospermes *Arch. Neerl.* III (B) I 146-234

14 Hooke J D 1872 "Flora of British India." London

15 Hum O.A. 1906 : Beitrage zur Morphologie und physiologie der Antipoden. B. Bot. Zbl. 20 : 77 174

16. H tchinson, J 1926 1959 : "The families of flowering plants 2 vols. London.

17 Johnson A M 1930 : "Taxonomy of th flowering plants. New York.

18. Lawrence G H M. 1951 : "Taxonomy of the vascular plants. MacMillan & Co. New York.

19 Lindley J 1853 "The Vegetable Kingdom. Bradbury & Evans, London.

20 Norris, T 1911 Torus anatomy and nectary haracteristic as ph l genetic in th Rhoedales. *Amer J Bot.* 28 : 101 113.

21 Puri V 1951 : The ole of floral natomy in the solution of morphological problems *Bot Rev* 17 : 471 553.

22. Rendle A B. 1925 "The classification of Flowering plants. Vol. II Cambridge

23 Saksena H B 1954 Floral morphology and embryology of *Fumaria parviflora* Lam. *Phytomorphology* 4 : 409-417

24 Saunders E R. 1932 O some recent contributions nd criticism dealing with morphology in Angiosperms. New Phytol. 31 : 174 19

25. Sa nd rs, E R 1937 : Floral Morphology a new outl k. I. Cambridge

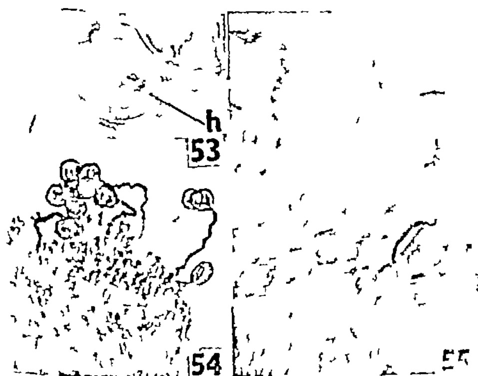
26 Schnarf K 1931 Vergleichende Embryologie der Angiospermen. Berlin

27 Souèges R 1911 Embryogénie des Fumariacees. L origine d corps de l'embryon chez le *Fumaria officinalis* L. C R A S. Pa. 213 : 527 531

28. Souèges, R. 1916b Embryogénie des Fumariacées La différence fondamentales d corps ch z le *Fumaria* O f a. L C R Acad. Par 213 : 699-701

29 Souèges, R. 1916a Embryogénie les Fumariacées Les premiers termes d developpement de l embryon l le *Cerydalis* Lutea DC. C R A S Par 222 161 163

30. Souèges R 1916b : Embryogénie des Fumariacées La differences fondamentales du corps che le *Corydalis* lutea DC C R Acad Sci Paris 222 : 753 755


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SHARPNESS OF THE COLLOID-ELECTROLYTE BOUNDARY AS A CRITERION TO MEASURE THE MOBILITY OF COLLOID MICELLES. PART I ELECTROPHORESIS OF $Fe(OH)_3$ SOL

P D BHATNAGAR AND ABANI K. BHATTACHARYA

In a previous communication the movement of the colloid-electrolyte boundary of $Fe(OH)_3$ with various concentrations of KCl as supernatant liquid was considered in the light of Kohlrausch relation $T_A/C_A = T_B/C_B$ for the ionic boundaries.

The present paper deals with the sharpness of the colloid electrolyte boundaries of $Fe(OH)_3$ sol with various equiconducting supernatant electrolytes viz. LiCl, NaCl, KCl, $BaCl_2$ and $AlCl_3$. The colloid-electrolyte boundary can be regarded analogous to an ionic boundary only to a certain extent. Colloid-electrolyte interaction, not completely understood at present, appears to be responsible for the divergence. It has been discussed that a more probable value of the electrophoretic velocity of a colloid micelle can be obtained by measuring the movement of the descending boundary when the colloid particles behave like the leading ions.

EXPERIMENTAL

The apparatus consists of two parts after Tiselius pattern, the electrode vessel and the main U tube fitted by standard glass joints at P_1 and P_2. M_1 and M_2 are the electrodes supplying a constant current the device for maintaining a constant current is described below :—

A sharp-cutoff pentode 6 S J 7 whose plate current is independent of its plate voltage within a range of 40 to 500 volts (R. C. A. Receiving tube manual p. 0 211) is used in series with the U tube as shown in the circuit.

The voltage drop across the whole U-tube is first of all determined by the potentio—V T V M system allowing the required current (0 1 to 0 4 milliamps) to flow through the circuit. The supply voltage from the rectifier is so adjusted that the plate voltage remains sufficiently above 40 volts. Now because of the characteristics of the tube 6 S J 7 any change in the voltage drop across the U-tube due to changes of the resistance will not change the current in the circuit as long as the plate voltage remains between 40 to 500 volts. The grid of the valve is connected to a series of batteries to give the required negative potential for the desired amount of current (0 1 to 0 4 milliamps)

OBSERVATIONS AND DISCUSSION

Mukherjee (1928) Henry and Brittain (1933) and others observed that in the electrophoresis of colloids studied by the moving boundary method in a Bur-

ion a U tube, the sharpness of the boundary depended upon the relative velocities of leading ion and of the colloid micelles. According to Henry and Brittain the ascending boundary becomes sharp when the colloid particles have a lower velocity than the leading ions while the descending boundary becomes diffuse because the faster ion in the ascending limb has now to follow the colloid particles in the descending limb This effect which was observed initially for the ionic boundaries was also reproducible in the colloid-electrolyte boundary movements under the applied potential gradient. The sharpness and diffuse nature of the boundaries in the ascending and descending limbs have been depicted in the plate nos. 1 & 2.

With the equiconducting solutions of the chlorides of Na, K, Ba & Al, it will be seen (Plate no 1) that the descending boundary becomes more and more diffuse as the electrophoresis proceeds while with LiCl solution as supernatant liquid the descending boundary acquires sharpness with time (Plate no

TABLE 1

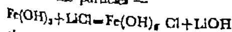
Equiconducting Supernatant liquid	Ascending boundary LiCl	NaCl	KCl	$BaCl_2$	$AlCl_3$	Descending boundary LiCl	NaCl	KCl	$BaCl_2$	$AlCl_3$
Mobility of the cation	39	56	73	63		39	56	73	63	
$Fe(OH)_3$ Sol boundary	Diffuse (tends to sharpness after some time)	Sharp	Sharp	Sharp	Sharp	Sharp	Dif.	Df.	Df	Df
Plate No	1	2	2	2	2	1		2		1
Observed mobility of $Fe(OH)_3$ micelle	30	93	41	58	60	41	2[illegible]6	18	33	3[illegible]

(Temperature 25°C)

The foregoing observations can be interpreted qualitatively by the relative mobilities of the leading and the indicator ion theory as proposed by Kohlrausch and Weber for ionic boundaries According to this theory the essential condition for a stable boundary is that a slower moving ion follows a faster moving ion such that both move with equal speed when the [illegible] is reached The ionic mobilities of Li^+, Na^+, K^+ and Ba^{++} are [illegible] 56, 73 and 63 respectively as shown in the table 1 [illegible] K^+ and Ba^{++} chlorides as supernatant liquids the descending boundary of the $Fe(OH)_3$ sol becomes diffuse while the ascending one [illegible]

sharpness, it follows, according to this theory that the mobility of $Fe(OH)_3$ micelles, is lower than the Na^+, K^+ and Ba^{++} ions.

With LiCl as supernatant liquid the boundary in the descending limb becomes sharp indicating that the $Fe(OH)_3$ micelles which assume the role of leading ions have a greater mobility than the indicator Li ions. The tendency of the ascending boundary of $Fe(OH)_3$ LiCl to acquire sharpness violating the Kohlrausch-Weber theory may be due to colloid electrolyte interactions. Such a tendency has been observed (see plate no. 1) in which diffuse nature was gradually suppressed. This kind of observation may be due to the change of the species of colloid particles constituting the boundary. It will be seen in plate no. 2 that two sharp ascending boundaries tend to merge together after about 60 minutes. The probable mechanism of this behaviour may be due to the change in the $Fe(OH)_3$ micelle during their movement under the applied potential of the field. The colloidal micelles may interact with LiCl as follows changing the nature of the particles :—



$$Fe(OH)_3 + LiCl = Fe(OH)_2 Cl + LiOH$$

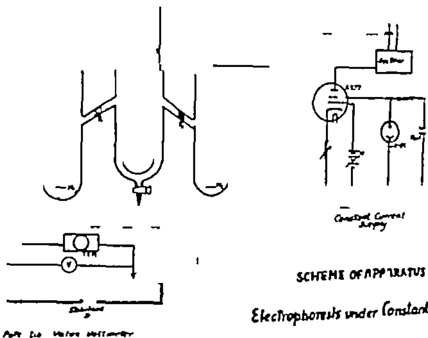
The sharpness in the ascending boundary may result from the slower $Fe(OH)_2Cl$ micelles following the comparatively faster micelles of $Fe(OH)_3$.

Thus we may conclude from these observations that a descending sharp boundary should enable us to determine the mobility of the colloidal particles more accurately or at least it will be the more representative value of the micelle velocity when the colloid micell are made to behave as leading ions. For positive sols this can be achieved by choosing a suitable cation whose mobility is lower than that of the colloidal micelle producing sharpness in the descending limb. This is supported by the electrophoretic velocity of $Fe(OH)_3$ particles when the sharp boundary is formed in the descending limb with LiCl as the supernatant liquid. The values calculated from the sharp boundary in the ascending limb correspond to the mobility of the leading ions rather than the actual mobility of the colloidal micelle.

Further work is in progress.

REFERENCES

1. Henry D. C. & Brittain John : Trans. Faraday Socity 29 798 (1933)
2. Mukherjee J. N. : Proc. Roy. Soc. A, 102 103 (1923)



SCHEME OF APPARATUS

Electrophoresis under Constant

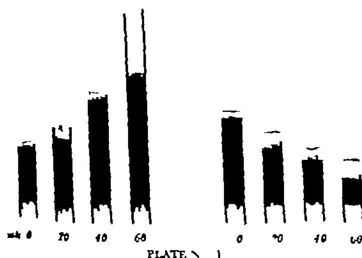


PLATE No. 1

Ascending boundary *Descending boundary*

$Fe(OH)_3$ Sol—Equiconducting NaCl supernatant Liquid

Similar results with KCl, $BaCl_2$, $AlCl_3$ equiconducting Solutions

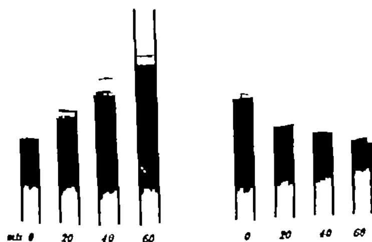


PLATE No. 2

Ascending boundary *Descending boundary*

$Fe(OH)_3$—Equiconducting LiCl supernatant liquid

STUDIES ON THE DIGESTIVE SYSTEM OF *SCARITES INDUS* OLIVIER (COLEOPTERA : CARABIDAE)

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INTRODUCTION

The study of the Morphology of *Scarites indus* Olivier was undertaken because it is a very common "Ground beetle" abundantly found in this locality (Agra). Termites form its chief food, that is why they are abundantly met with in termite infested sugarcane stubbles. Under adverse circumstances these insects become cannibalistic in nature.

The present paper deals with the digestive system of these insects about whom not much is known except in certain allied forms viz., *Calosoma sycophanta* Linn. (Bess, H. A. 1935) *Anopheles menoncalus* Hbst., (Bigham, J. T. 1931) and *Harpalus pennsylvanicus* Dej. (Whittington, F. B. 1935).

MATERIAL AND TECHNIQUE

Beetles were generally collected in early morning hours from various localities in Agra and kept alive in vivarium by artificial feeding. This formed a continuous source of supply for the author's study. Dissections were performed in freshly killed specimens under normal saline solution by the help of a stereo-scopic binocular microscope. Most of the sketches were prepared with the help of camera lucida while gross anatomy was sketched free hand.

MOUTH PARTS

The mouth parts are of biting and chewing type and consist of the usual components.

Labrum. Figure No. 1 (a)

The labrum is rectangular in shape, 1/3rd long as broad, with antero-lateral margins convexly outstretched and provided with 14-16 stiff setae together with a single long bristle directed forwards and a little inwards on either side. The anterior margin is further prolonged in the middle into a bluntly truncate lobe but devoid of any setae. Its posterior margin is buttressed by a strong apodeme (*Tormae* of Snodgrass) which sends out short stumpy processes at the postero-lateral angles for the insertion of stout muscles which arise from the frons. A loose membrane running along the labial suture further connects it to the clypeus.

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Mandibles Figure No. 1 (b, b')

Each mandible is a stout heavily chitinised structure with convex outer denticulate inner and triangular basal margins. There are two teeth in the right mandible and three in the left, arranged so as to fit into each other

It articulates with the cranium by the help of a condyle situated at the postero-lateral outer angles close to the insertion of the highly developed abductor muscle. The two mandibles are brought together in close apposition by a large stout powerful adductor inserted on the inner angles of each of the mandible Both these muscles originate from the lateral walls of the cranium.

Maxillae Figure No 1 (c, c')

Each maxilla has usual components and is articulated with the head capsule at the gular suture by the cardo-condyle The lacinia is long narrow inwardly fringed with stout setae at the apex and a number of small setae at its inner margin The galea is elongate and is articulated at its middle A four jointed maxillary palp is attached to the base.

Three muscles are inserted on the cardo Ventral flexor muscle of the maxilla originates from the tentorium the dorsal flexor from the cranial capsule and partly from the tentorium while the extensor muscle arises from the cranial wall only There is also another muscle (tentorial flexor of the stipes) which arises from the inner margin of the tentorium and is inserted basally at the stipes.

Labium Figure No. 1 (d)

The labium has a triangular proximal prementum, a small rectangular submentum lying between the bases of the two maxillary cardines and mentum. Mentum is large and somewhat rectangular anteriorly truncate plate beset with a few bristles and forms the floor of the mouth.

A pair of levator muscles originating from the tentorium and extending forward and inwards are inserted on a long common apodemal inflexion at the base of the pre-mentum A pair of parallel muscle bundles arises from the apodemal inflexions at the base of the post-mentum and slightly extend mesad to be inserted basally on the pre-mentum They serve as retractors of the prementum A pair of flexors of labial palpi arise from the basal sclerotised rich of the post mentum and are inserted basally on the palpus inwards.

A two jointed labial palp along with a palpiger is attached to the labium outside the ligula—a broad flap like structure lying between the maxillary palps and shows no differentiation between glossa and para-glossa

ALIMENTARY CANAL

(Fig No. 2)

The alimentary canal of *Scant s* ... runs along the middle of the ... through thick muscles of the thorax and fatty tissue of the abdomen and a

heavy mass of tracheoles. It is slightly longer than the body length. Except for three convolutions in the third and fourth abdominal segments it is nearly straight. It is associated with four malpighian tubules and a pair of rectal glands.

Stomodaeum

The mouth, bounded by the mouth parts, leads into a spacious buccal cavity which narrows posteriorly into a pharynx which transmits food material into the crop through an elongated tubular oesophagus mainly located in the thorax. The salivary glands are absent in this insect

The crop is a large membranous sac situated in the metathorax and first abdominal segment. It can be easily differentiated from the following bulbous proventriculus owing to the presence of sphincter muscles. It leads to the conspicuous mesenteron or ventriculus bounded anteriorly by a proventricular valve and a pyloric valve at the posterior end. There is a gradual reduction in its diameter posterads. It appears as a slightly tortuous tube invested with closely packed small villi or enteric caecae diminishing in size and number towards the posterior end. In the hibernating forms the crypts are small and finger like but in the actively feeding individual they are long and bulbous at the bases. It is in this region, that the major role of digestion is played.

Proctodaeum

The rest of the alimentary canal posterior to the sharply marked off constriction of the pylorous is the proctodaeum which is located in the fifth abdominal segment. The first thin walled narrow anterior portion may be called ileum and the broad thin walled posterior region which opens to the outside—the rectum. It is provided with a sphincter muscle for the ejection of faecal matter

Malpighian Tubules

Malpighian tubules are four in number and arise all round the pylorus in the fifth abdominal segment. They are long slender unbranched tubes, which form numerous coils on the alimentary canal. They are creamy white in colour at their bases turning brownish towards their free ends, which float about freely in the body cavity They first run anteriorly forming several coils round the ventriculus and then descend into the rectal region and once again run forwards to form dense coils. After ascending a little distance they lie freely in the haemocoel without any reassociation All along their course the malpighian tubules are traversed by minute tracheoles.

GLANDS

Salivary glands are absent In the anal region however is present a pair of anal glands. It is composed of a glandular region followed by a slender duct which open into a sac like vesicle. From this vesicle leads a short and stout duct to opens in the rectal region. This gland secretes some fluid which is probably used for defensive purposes by these insects.

Summary

1 Mouth parts are adapted for carnivorous habits.
2 Food chiefly comprises of termites, earthworms etc., and during adverse conditions they exhibit cannibalistic habit.
3 Salivary glands and hepatic caecae are absent. Anal glands present.
4 Malpighian tubules are only four in number

Acknowledgements

The author is highly indebted to Dr R D Saxena Professor and Head of the Department of Zoology B. R. College, Agra, for suggesting the problem and also for his unstinted help and guidance during the course of investigation. Thanks are also due to Dr R. K. Singh, Principal, B. R. College Agra for providing necessary facilities for work, conducted in the laboratories of the Zoology Department in partial fulfilment for the M Sc. degree of the Agra University

References

1 Andrews, H. E. (1929) : The Fauna of British India including Ceylon and Burma Coleoptera, Carabidae. 1 Carabinae.

2. Bess, H. A. (1935) : Alimentary canal of *Calosoma sycophanta* L., *Ohio J Sc* 35 (1) : 54-64

3. Bigham, J T (1931) : Alimentary canal of *[illegible]* Hbst. *Ohio J Sc.* 31 (5) : 386-393

4 Snodgrass, R. E. (1935) : Principles of Insect Morphology McGraw Hill Book Company Inc., New York & London.

5. Whittington, F B. (1935) : Alimentary canal of *Harpalus pensylvanicus* Dej. (Carabidae : Coleoptera). *Ohio J Sc.* 35 (2) : 131-138

Fig No I

(Structure of th mouth parts and their musculature) *abd* abductor muscle *add*: Labrum; *b, b'* Mandibles *c'* Maxillae; *d* Labium *abd* abductor muscle *add* ; Labrum; *b, b'* Mandibles *c'* Maxillae; *d* Labium. *Em* extensor of abductor muscle; *C* cardo; *Con* condyle; *Dfl* dorsal flexor of maxilla *Em* extensor of ... *IL.Max* inner lobe of maxilla; *fl Lab p* flexor of labial palp; *fl. st* flexor of stipes *Lg* Ligula = mentum; maxilla (lacinia); *Lab. p* labial palp *Lev.m* levator of labium *Pm* prementum *Pro* *Max. p* maxillary palp *Ol. Max* outer lobe of Maxilla (galea) *Rs Pm* retractor muscles of pre protractor muscles; *Rs* retractor muscles of labrum; ... mentum; *Sm* submentum; *St* Stipes *Vfl* ...

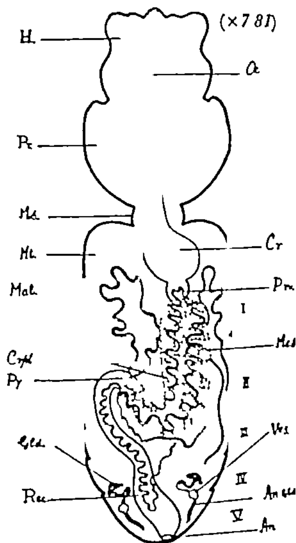


Fig No 2.

(Dorsal view of the digestive system)

An anus *An. Gld*: anal gland; *Cr* crop *Crpt*: crypts; *Gld*: gland; *H*: head, *Mal* malpighian tubules *M* : Mesentron; *M* mesothorax *Mt*: metathorax; *Oe* oesophagus *Pr* prothorax; *Pro*: proventriculus *Py*: pylorus *Rec*: rectum *Ves* vesicle I to V visible abdominal segments.

STUDIES ON FLORAL BIOLOGY OF PHALSA (*GREWIA ASIATICA* L.)

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Grewia asiatica L. (Phalsa) is a member of the Tiliaceae family This fruit is probably a native of India and grows well on the plains. These days, its juice is highly esteemed by the people as it makes a cool and refreshing drink in the summer No information is available on the blossom biology and fruit-set of phalsa, so the present study was taken up

Phalsa with large fruits are locally called Sakari and those with small fruits are known as Sarbati. To study the floral biology four plants of each of the varieties were selected at the Botanical Garden, Government Agricultural College, Kanpur in the year 1961 These plants had received the same treatment and were of the same age and vigour

The floral buds are yellowish in colour before they open. In most cases it was observed that the calyx separated from each other at the sides about 14 to 16 hours before the anthesis but they were attached at the apex while in a few cases they separated on the day of anthesis. It takes about 10 to 30 minutes for full opening of the flower

To determine the time of anthesis floral buds were tagged and the number of flowers which opened between 7 a. m. and 12 noon were recorded at hourly interval. As soon as the flower opened, one of its petals was removed to avoid confusion in counting The data on Sarbati variety is presented in table I

It is clear from the table that the maximum number of flowers opened between 8 a. m. and 9 a. m. though they began to open from 7 a. m. and continued till 10 a. m.

Dehiscence

(*a*) *Mode of dehiscence* The transverse sections of the anthers showed that there are two layers of pollen sac walls. At places where there is only a single layer of cells the endothecium ruptures from base towards top probably due to the effect of humidity heat or internal pressure of pollen grains.

The anthers of the outer whorls in both the varieties dehisced first but those which were small in size did not dehisce and dried. It was also noted, that the length of filaments varied from 0 4 to 0 9 cm. The anthers of the outer whorl are generally larger than the inner ones. At the time of anthesis anthers are yellow in colour and an hour after the dehiscence they withered and turned red after 3 to 4 hours of anthesis.

TABLE 1

Time of opening of flowers in Sarbati variety of Phalsa.

Date of observation	Percentage of flowers opened at hours					Total number of flowers record
	7–8 a.m.	8–9 a.m.	9–10 a.m.	10–11 a.m.	11 a.m. 12 noon	
11-4-61	10.7	60.1	29.2			92
12-4-61	11.3	61.7	21.0			107
13-4-61	14.2	66.2	19.6			118
14-4-61	9.0	72.1	18.5			107
15-4-61	10.5	62.8	26.7			109
16-4-61	10.3	61.5	8.4			114
17-4-61	13.2	67.8	19.0			121
18-4-61	13.7	67.9	18.4	—		103
19-4-61	14.1	67.9	18.0		—	109
20-4-61	15.4	68.5	16.1			115
21-4-61	16.7	69.0	14.3			178
22-4-61	16.9	69.0	14.1		—	132
23-4-61	14.2	70.7	15.1			110

(b) *Time of dehiscence*. It was observed that a few large sized anthers dehisced even before anthesis but in general they dehisced after anthesis.

Pollen Studies :

(a) *General appearance and shape of the pollen.* The pollen grains appeared as a fine yellowish powdery mass and remained in the anthers unless these were disturbed. The pollen grains were stained in methyle green glycerine jelly (Wodehouse 1935)

The pollen grains are not of uniform shape and size but some of them appeared deformed—cylinderical, shrivelled, and twisted also however the normal grain looked round (Fig 1) In acetocarmine, the exine could be clearly seen with the germpores The exine is fairly thick, transparent and has a net work like structure. The number of germpores varied from 1 to 4 per pollen grain.

(b) *Pollen size.* The size of pollen of Phalsa was measured under high power The average size of 50 pollen grains in each variety was noted and it was found to be 56μ in Sarbati variety and 50μ in Sakari variety

(c) *Pollen fertility* The fertility of the pollen grains was studied in acetocarmine and on the flowers with stigma of different ages Deeply stained and normal pollen grains were taken as viable ones, while shrivelled and non-stained pollen grains were counted as non-viable (unfertile) Pollen grains were counted in different fields of the slides. The observations on pollen viability are given in table 2.

TABLE 2

Pollen fertility in the varieties of Phalsa

Variety	Total number of pollen studied	Number of fertile pollen	Number of sterile pollen	Percentage of fertile pollen
Sakari	987	674	313	68.29
Sarbati	1026	789	237	76 81

From the above table it is clear that the percentage of viability was 60 29 in Sakari and 76 81 in Sarbati

(d) *Artificial germination of pollen grains* Pollen germination was studied in different concentrations of sugar solution—5 10 15 20, and 25 per cent—with 1 5% agar at room temperature.

Not less than 650 pollen grains were studied in each concentration and 20% sugar solution with 1 5 per cent agar gave the highest percentage of

germination Sugar solutions of 10 and 15% concentration in both varieties gave lower percentage of germination than 25 per cent. The data are summarized below

TABLE 3

Percentage of pollen germination in different concentration of sugar-agar media.

Variety	Sugar concentration in the media				
	5	10	15	20	25
Sakari		4 5	20 5	71 4	31 1
Sarbati	..	6	4	79 6	4 2

Pollen Studies II

(*a*) *Recepticity of Stigma*—Controlled pollination on flowers which were emasculated 12 hours before anthesis and bagged, was made at different intervals—from 2 days before anthesis to the day of anthesis Further pollination was not done because the stigmas started drying one day after anthesis. 40 flowers were pollinated under each treatment. Utmost care was taken in all the operation—selection of flower buds, emasculation, pollination, bagging, labelling etc

At the time of pollination the external condition of the stigma was also observed. The stigmas were pollinated with pollen grains from freshly dehisced anthers. Out of the bagged and pollinated flowers a few stigmas were taken at random and studied under the microscope after necessary maceration treatment For determining the percentage of pollen germination of stigma, they were fixed at various intervals after pollination i e., 12 18, 24 36 and 48 hours.

The stigmas for examination of pollen growth were fixed in aceto-alcohol (1.3) for 10 minutes. These were then preserved in 70% alcohol in order to prevent hardening until they could be examined. The following process was adopted for staining and maceration

(1) The styles which were previously fixed in aceto-alcohol (1.3) were stained with lactophenol after maceration (Darlington and La Cour 1947)

(*b*) *Duration of Recepticity of Stigma*—The stigma becomes receptive 24 hours before anthesis and maintains its recepti[illegible] up to 24 hours after anthesis. After about 30 hours of anthesis the stigmas became dull green and the styles began to dry up and eventually dropped off if pollination had not taken place. This was confirmed by the study of controlled pollination of emasculated flowers. Data are presented in table 4

TABLE 4

Receptivity of stigma in varieties of Phalsa as judged from the percentage of fruit set the number of flowers pollinated in each case being 0

Varieties	36 hours before	24 hours before	12 hours before	At thesis	12 hours after	24 hours after	36 hours after
Kalari	0	45	59	84	34	0	0
Sarbati	0	56	78	96	43	2	0

(c) *Germination f pollen on the stigmatic surface*—The germination of pollen grains on the stigmatic surface was also observed. Table 5 also shows that the stigma becomes receptive 24 hours before anthesis and remains so till 24 hours after it. However after 24 hours of anthesis only 4·2% of stigmas shows pollen germination It is also clear from table 5 that the stigma has maximum receptivity at the time of anthesis and prior to anthesis but it decreases after the opening of the flower At the time of anthesis the pollen germination was 87·5% while 12 and 24 hours before anthesis it was 70·8 and 58·3 per cent respectively

POLLINATION AND FRUIT SET STUDIES

The different phases of pollination in Sarbati variety were studied and they are as follows

(1) *Self Pollination.* 56 selected flowers were bagged one day prior to anthesis in the Sarbati variety only

(2) *Hand Pollination.* 66 flowers were emasculated and bagged 10 hours before anthesis. When the flowers fully opened the stigmas of the emasculated flowers were pollinated with the pollen of the freshly opened flowers. After pollination these flowers were carefully bagged again

(3) *Natural open Pollination.* 76 flowers were tagged and left as such for the open pollination They were observed for subsequent fruit setting

(4) *Natural cross Pollination.* 10 hours prior to anthesis 63 flowers were emasculated and left to be pollinated by natural agencies—bees, flies insects and wind Thus natural cross pollination was determined. The number of fruit set was recorded under all the four modes of pollination after 10 days of pollination and observations are presented in table 6.

TABLE 5

Pollen germination on stigma in Phalsa (Sarbati Variety)

Sl No.	Stigmatal age in relation to anthesis	Stigma examined after hours of pollination												Stigmatal Receptivity		
		6 hours			12 hours			18 hours			4 hours			Total stigma studied	Stigma with pollen tube	Percentage of receptive stigma
		a	b	c	a	b	c	a	b	c	a	b	c			
1	36 hour before	6	0	0	0	0	0	6	0	0	6	0	0	24		
2	24 hours before	1	2	0	3	3	0	2	4	0	1	4	1	24	14	58 3
3	12 hours before	3	3	0	2	3	1		2	2	0	2	4	24	17	70 8
4	At anthesis	3	3	0	0	3	3	0	2	4	0	1	5	4	21	87 5
5	1 hour after	5	1	0	4	1	1	3	2	1	3	2	1	24	9	37 5
6	[illegible] after	6	0	0	6	0	0	6	0	0	5	1	0	24	1	4 2
7	6 hours after	6	0	0	6	0	0	6	0	0	6	0	0	24	..	

[illegible] germination of pollen.
[illegible] germination of pollen.
[illegible] good germination of pollen.

TABLE 6

Effect of different methods of pollination on fruit set in Sarbati variety

Sl. No	Methods of pollination	Number of pollinated flowers	Number of fruits set in	Percentage of fruit set
1	Self pollination	56	37	66 07
2.	Hand pollination	66	54	81 82
3	Natural open pollination	76	55	72 36
4	Natural cross pollination	63	34	53 96

The above table shows that the maximum fruit setting (81 82 %) was found in the hand pollinated flowers followed by natural open pollination self pollination and natural cross pollination Thus t is clear that of all the methods of pollination, hand pollination increased the percentage set of fruits.

The data on the composition of 50 fruits are summarized in table 7

TABLE 7

Composition f fruits

Varieties	Average diameter of fruits in cm.	Average weight of pulp per fruit in gm.	Average weigh of seed per fruit in gm.	Percentage weigh of pulp in gm.	Percentage weight of seed in gm.
Sakari	1 42	1 324	0 086	90 68	9 32
Sarbati	1 10	0 670	0 080	88 10	11 90

The table shows that the fruits of Sakari variety were larger in size (1·42 cm.) than Sarbati (1 10 cm.) The average weight of pulp per fruit was 1·324 gm, while the percentage of the edible pulp per fruit was 90 68 in Sakari variety

DISCUSSION

In the present studies, it was observed that anthesis in Phalsa commenced early in the morning but maximum number of flowers opened between 8 a. m to 9 a. m. Similar observations have been reported by Sen et al. (1946) in mango The maximum dehiscence of anthers took place before anthesis though in some cases they dehisced just after anthesis

Studies on pollen fertility revealed that it was higher (76 18%) in Sarbati variety while Sakari had only 68 29 per cent fertility Pollen germination in both the varieties was studied and the maximum germination was found in 20 per cent sugar solution with 1·5 per cent agar It was also found that in lower concentrations (0% and 5%) of sugar pollen grains swelled and at times they burst opened.

Stigma receptivity was maximum prior to anthesis and it decreased after opening of flowers And as the maximum dehiscence of anthers takes place prior to anthesis so it is helpful in pollination and fertilization of the flowers.

Morphological features of pollen grains were similar in both the varieties however the size of pollen grains of Sarbati variety was bigger (56μ) than the Sakari (50μ)

The present study revealed that it was possible to obtain as high as 81 82 per cent of fruit set in Sarbati variety by hand pollination and this brings out clearly the need for pollination and pollinating agents in Phalsa.

Average fruit size, weight and pulp to seed ratio in terms of percentage were greater in variety Sakari than the Sarbati variety

Summary

1 The floral biology of phalsa was studied in two varieties namely Sarbati and Sakari in the year 1961

2. The opening of flowers is completed in a very short time in both the varieties.

3 Dehiscence of anthers took place just prior to and at the time of anthesis. The anthers of the inner whorl are small in size and did not dehisce but dried later on

4 The pollen grains in both the varieties were similar in shape however the size of the pollen grain was 56 in Sarbati and 50 in Sakari variety

5 The maximum pollen germination was observed in 20% sugar solution with 1 5 % agar

6 The stigma was found to be receptive from 24 hours before anthesis to 24 hours after anthesis

7 The maximum percentage of fruit set (81 82) was found in hand pollination and minimum set (53·96) in natural cross pollination

8. The average percentage of pulp was 90 68 and seed percentage was 9·32 in Sakari variety

Literature Cited

1. Darlington, D.C. & L.F. La Cour 1947 : Handling of chromosomes. George Allen & Unwin, London.
2. Sen, P.K., Mallik, P.C. & Ganguly B.D. 1946 : Hybridization of the mango. *Indian J. Hort.* 4 (1 and 2) : 4-15.
3. Wodehouse R. P. 1935 Pollen grains. Mc Graw Hill. Company New York.

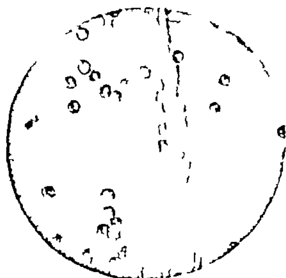


Fig. 1. Showing Pollen grains in ... carmine

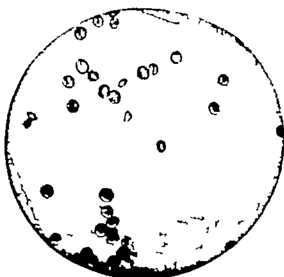
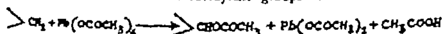


Fig. 1 Showing Pollen grains in aceto carmine

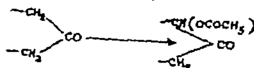
SOME RECENT OBSERVATIONS OF LEAD TETRA ACETATE OXIDATION

M M Bokadia
Principal, Birla Govt Degree College Srinagar (Garhwal) U P

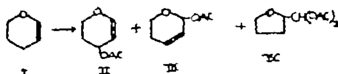
Lead tetra-acetate has found many diverse applications [1-3] in organic Chemistry It can oxidise active methylene groups as shown below



This oxidative reaction requires that the methylene group must be activated Such activation is possible when it is adjacent to certain groups e.g., a carbonyl group, a double bond, or an aromatic nucleus Thus simple ketones like acetone, diethyl ketone, acetophenone and cyclohexanone[4,5] readily form the acetate of the corresponding α-ketol on treatment with lead tetra-acetate Similarly the hydrogen of the methylene group of β-diketones and

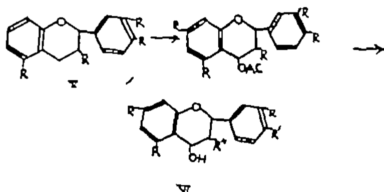


β-ketoesters[4,6] can be readily replaced by an acetoxyl group. The allyl position of olefins is also susceptible to lead tetra-acetate attack, although side products may result on account of interaction at the double bond or oxidation may be accompanied by rearrangement For instance cyclohexene[7] and α-pinene[8] furnish the normal reaction products while in the case of 5 6-dihydro-4H-pyran (I) Hurd and Edwards[9] obtained 3,6-dihydro-4H–pyran-4-yl acetate (II) 5 6-dihydro-2H pyran-2 yl acetate (III) and tetrahydrofurfurylidene acetate (IV)

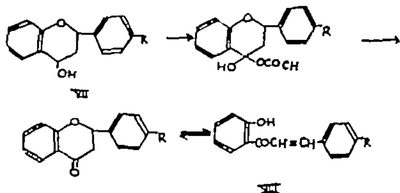


It is well known that lkyl side chains attached to aromatic ring systems are specifically activated towards this oxidation at the position adjacent to the aromatic nucleus. Typical examples are the oxidation of toluene[4] ethyl benzene[10] tetralin and 6-methoxytetralin An elegant application of this principle consists in the insertion of a hydroxyl group on the C (4) of a flavan molecule (V)[13] However the yields of 4-ols drop considerably as the flavan nuclei are increasingly substituted by the methoxyl groups. Thus only a 10 yield of (+)3' 4 ,3,7-tetramethoxyflavan-3, 4-diol ((+)-leucocyanidin tetra methyl

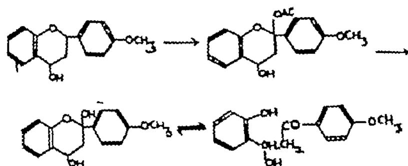
ether) is obtained in the case of (+)-catechin tetra methyl ether (V R=R =OCH_3, R =OH) In a highly methoxylated flavan molecule the C(7) and the anionoid nuclear carbon atoms provide alternate site for acetoxylation.



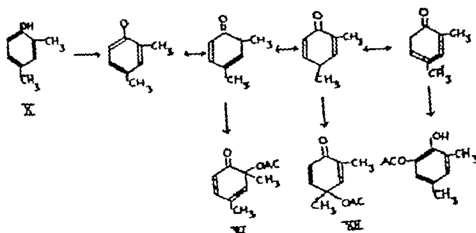
The lead tetra-acetate oxidation product of flavan-4β-ol (VII) yielded on hydrolysis 2 hydroxychalcone (VIII) in 40%.



This shows that, with excess of the reagent the 4-position of flavan undergoes disubstitution. Disubstitution at the same carbon atom has also been observed by Cavill, Robertson and Whalley[11] in the lead tetra-acetate oxidation of fluorence xanthene 1 7-d methoxyxanthene and 2,3-dimethoxyxanthene. On the other hand in the lead tetra-acetate oxidation of cyclohexanone Cavill and Solomon[4] found that the primary oxidation product, 2-acetoxy-cyclohexanone is further oxidised at a second active centre to give 2 6-diacetoxy cyclohexanone and they did not observe any disubstitution at the same carbon. However when 4 methoxyflavan-4β-ol (VII R=OCH_3) was oxidised with lead tetra-acetate the oxidation product on alkali hydrolysis and acid treatment yielded a complex mixture presumably of (VIII R=OCH_3) and (IX) arising from attack at C(4) and C(?) respectively Attack on C( ) would form [illegible] after hydrolysis of the oxidation product, ? 4-d hydro[illegible] methoxyflavan, which would exhibit ring-chain tautomerism[11] and in the presence of [illegible] (IX completely change to a β-d hydro-β 2-dihydroxy-4-methox[illegible]

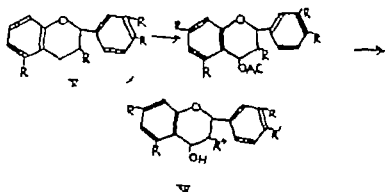


Recently Wessely and co-workers[18] and Cavill, Cole Gilham and Mc Hugh[19] have studied the lead tetra-acetate oxidation of free phenols. The products obtained vary with (a) the solvent, (b) the proportion of the oxidising agent and (c) the position and number of CH_3 substituents. The first step of the reaction is the formation of a phenoxide radical produced by dehydrogenation of the phenol. Then dimerization occurs in benzene solution but in acetic acid which assists the propogation of acetate radicals, acetoxylation predominates. Phenol itself affords only a dimeric product (4 4 -dihydroxydiphenyl) but with increasing o, p-substitution this reaction slows down. Thus in mesitol only acetoxylation occurs irrespective of solvent. Attack by acetate radicals at substituted o-and p-positions stabilises the dienone system. For instance 2,4-dimethyl phenol (X) yields (XI) and (XII)

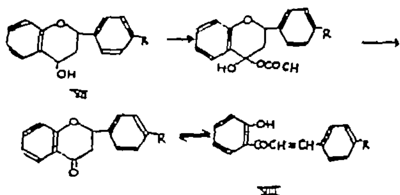


However a series of products has been obtained by the lead tetra-acetate oxidation of phenol ethers. With 1,3,5-trimethoxybenzene (XIII) Bokadia Brown and Cummings[8] isolated nearly 1% of nuclear acetoxylated compound 2,6-dimethoxybenzo-1 4-quinone (XIV) and 1 5-dimethoxy 5-acetoxybenzene (XV). Preuss and Menzel[17] is reported simila results for such oxidation of p-dimethoxybenzene and eratrole

ether) is obtained in the case of (+)-catechin tetra methyl ether (V R=R =OCH_3, R =OH) In a highly methoxylated flavan molecule the C(7) and the anionoid nuclear carbon atoms provide alternate site for acetoxylation.



The lead tetra-acetate oxidation product of flavan-4β-ol (VII) yielded on hydrolysis 2 hydroxychalcone (VIII) in 40%



This shows that with excess of the reagent, the 4 position of flavan undergoes disubstitution Disubstitution at the same carbon atom has also been observed by Cavill, Robertson and Whalley[13] In the lead tetra-acetate oxidation of fluorence xanthene 1 7-dimethoxyxanthene and 2,3-dimethoxyxanthene On the other hand in the lead tetra acetate oxidation of cyclohexanone Cavill and Solomon[8] found that the primary oxidation product 2-acetoxycyclohexanone is further oxidised at a second active centre to give 2,6-diacetoxycyclohexanone and they did not observe any disubstitution at the same carbon. However when 4 methoxyflavan-4β-ol (VII R=OCH_3) was oxidised with lead tetra-acetate the oxidation product on alkali hydrolysis and acidification yielded a complex mixture presumably of (VIII R=OCH_3) and (IX) arising from attack at C(4) and C(?) respectively Attack on C(?) would furnish after hydrolysis of the oxidation product 2,4-dihydroxy 4-methoxy flavan which would exhibit ring-chain tautomerism[14] and in the presence of alkali ... completely change to a β-dihydro-β 2-dihydroxy 4-methoxychalcone (IX

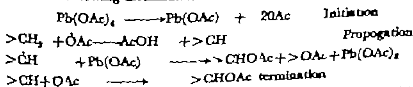
4' Methoxyflavan-4β-ol was prepared by Somerfield[13] by Lithium aluminium hydride reduction of 4-methoxyflavanone. The compound m. p. 144-145 obtained by Karrer *et al*[22] must be a mixture as these authors isolated both the 4α-ol and the 4β-ol from a similar reduction of flavanone itself.

Bognar *et al*[14] have observed that the flavan 3 4-diol obtained from dihydroflavonol (3-hydroxyflavanone) *via its* 4-amino compound has a 2(e) 3(e 4(ε) conformation. On this evidence, and with the assumption that a 3(e)) OH has not exhibited any steric control on the course of this reaction at C-4 the hydroxyl group in flavan-4α-ols may be assigned a quasi equatorial conformation. Some spectroscopic evidence has also been obtained in support of the above assignment. Infrared spectra of 4-methoxy flavan 4α-ol 4 methoxyflavan-4β-ol, and their acetates have been examined for this purpose. The α and β isomers have their (OH) absorption bands (in carbon tetrachloride, 0 0033M) at 3626 and 3616 cm^{-1} respectively indicating absence of hydrogen bonding. The C-O stretching frequency (in carbon disulphide, for the α-epimer is at 1019 cm^{-1} while for β it is at 1036 cm^{-1} (cf. 25 and 26) The C-O stretching frequency of the acetate of the α-epimer is at 1042 cm^{-1} (characteristic of equatorial acetates) while the corresponding bands for the acetate of the β-isomer are at 1062 and 1033 cm^{-1} (this splitting is characteristic of axial acetates)[13]

Based on the aforementioned evidence, the diol (VI $R = R = OCH_3$, $R = OH$) obtained from tetramethyl catechin is regarded as the 4α (quasi equatorial) epimer. From the known conformation of catechin [27,28] the conformation of this diol follows as 2(e) 3(e) 4(e') *. This conformation is further supported by preparing its isopropylidene devivative[3]

Mechanism of Lead Tetra-Acetate Oxidation

A free radical mechanism is generally accepted for this oxidation. Dewar[47] suggested the following mechanism.



At high temperature the reaction, however takes a different course and it brings about methylation of organic compounds[29,30]

In recent years evidence has been provided in favour of ionic mechanism of this oxidation[9,31] and the oxidising species is either CH_3COO^+ or $(CH_3COO)^+_2$.

* e denotes quasi-equatorial.

REFERENCES

1 Criegee, "Newer Methods of Preparative Organic Chemistry" Interscience Publ., New York, 1948, p. 1
2 Waters in Gilman's "Organic Chemistry" Vol. IV John Wiley 1953 p. 1120.
3 Criegee 1958 Angew Chemie, 70 173.
4 Dimroth & Schweizer 1923, Ber., 56 1375.
5 Cavill & Solomon 1955, Jour Chem. Soc 4426.
6 Goeber & Schwarz, 1951 Chem. and Ind 390.
7 Criegee, 1930 Annalen 481 263
8 Weis, Dissert, Karlsruhe 1953
9 Hurd & Edwards, 1954 J Org Chem., 19 1319
10 Cavill & Solomon, 1954 J Chem. Soc 3943
11 Johnson Anderson & Shelberg, 1944 J Amer Chem. Soc. 66, 218
12 Bokadia, Brown & Cummings 1960 J Chem. Soc 3308
13 Cavill, Robertson and Whalley 1949 Ibid., 1567
14 Grimshaw Johnson & King, 1958, Ibid. 4010
15 Wessely & Sinwel, 1950 Monatsch, 81 1055; 1953 84, 425 and 1951 85,69
16 Cavill, Cole Gilham & Mc Hugh 1954 J Chem Soc., 2785.
17 Preuss & Menzel, 1958 Arch. Pharm., 291, 350 & 377
18 Preuss & Tan, 1960 Ibid 65 505 & 517
19 Johnson, Gastambide & Pappo, 1957 J Amer Chem. Soc., 79 1991
20 Cavill, Dean, Mc Gookin Marshall & Robertson, 1954 J Chem. Soc., 4573
21 Freudenberg & Orthner 1922, Ber., 55, 1948.
22 Karrer Yen & Reichstein, 1930 Helv Chim. Acta, 13 1308
23 Bognar & Rakosi, 1957 Acta Chim. Acad. Sci. Hung 13 217
24 Bognar Rakosi, Fletcher Philbin & Wheeler 1959 Tetrahedron Letters 19 4
25 Allsop Cole White & Whillis 1956 Jour Chem. Soc., 4868.
26 Barton 1953 Ibid. 1027
27 Dewar "Electronic Theory of Organic Chemistry" Oxford Univ Press, 1949 p. 277
28 Fieser Clapp & Daudt, 1942 Jour Amer Chem. Soc 64, 2052.
29 Fieser & Chang, 1942, Ibid., 64. 2043.
30 Levitt 1955 J Org Chem. 20 1297
31 Bokadia, Brown Newbould Sommerfield & Wood 1961 Jour Chem. Soc. 4731
32 Bokadia, Brown Cobern Roberts & Somerfield 1962 Ibid 1658.

CONTRIBUTION TO THE EMBRYOLOGY OF *ARABIDOPSIS THALIANA* (GAY & MONN)

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INTRODUCTION

Arabidopsis thaliana, a member of the family Cruciferae, is an erect annual with petioled, obovate, and radical leaves. Flowers are small, white and grouped in racemes. Pods are very slender. The genus *Arabidopsis* consists of 11 indigenous species (Hooker 1873).

Although a lot of work has been done in the family Cruciferae, no one has paid any attention so far to the study of *A. thaliana*. This species has got an interesting feature for a breeder on account of its very short life cycle and also it can be grown at any time of the year under proper temperature control and in the suitable culture media in test-tubes.

MATERIAL AND METHODS

Flowers and fruits of different stages were collected and fixed in Formalin Acetic-Alcohol by Dr Bahadur Singh from Saharanpur and were kindly passed on to the author for investigation. Usual methods were followed for dehydration and embedding. Sections were cut at a thickness of 10-12μ. The slides were stained with Heidenhain's iron alum haematoxylin and destained with saturated picric acid.

MICROSPOROGENESIS AND MALE GAMETOPHYTE

In transections each of the four corners of a young anther shows hypodermal archesporial cells (Fig. 1). The cells undergo periclinal division and give rise to the primary parietal and primary sporogenous layer (Fig. 2). The former divides further essentially in periclinal plane so as to form three wall layers (Fig. 3). The outermost of these layers (next to the epidermis) develops into an endothecium and innermost into the tapetum, while the inner one forms the middle layer (Fig. 4) which disintegrates eventually. The tapetal cells at the beginning are uninucleate (Fig. 3) but later on, before the advancement of the meiotic division of microspore mother cells, they become binucleate (Fig. 4). Degeneration of the tapetum starts at the time of formation of microspore by the dissolution of its cell walls. Complete degeneration occurs when the pollen grains are bicelled. The endothecium acquires fibrous thickenings (Fig. 5). Finally at maturity only two layers of cells, an endothecium and some withering epidermal cells, represent the wall of the anther sac (Fig. 5).

The microspore mother cells start separating as meiosis advances. The divisions of the microspore mother cells are of the simultaneous type (Figs. 6 7 8 9 and 10) The second division of meiosis results in the formation of tetranucleate microspore mother cell (Fig 10) A mucilaginous outer wall surrounds it but soon its nuclei are separated forming four microspores which are either arranged tetrahedrally (Fig 11) or isobilaterally (Fig 12).

The microspore when first formed are uninucleate (Fig. 13). They soon acquire an exine closely appressed against intine. The single nucleus of the pollen-grain then divides to form a large vegetative and a small generative nucleus separated from each other by a fine cell-membrane (Fig 14) Before shedding the generative cell again divides to form two male cells. By the time of shedding the pollen-grains are tricolporate spheroidal and tricelled (Fig 15)

Megasporogenesis and Female Gametophyte

Ovule—There are numerous, anatropous, tenuinucellate bitegmic ovules. The primordia of both the integuments are laid down by the time of formation of megaspore mother cell (Fig 16) Growth of both the integuments is uniform for sometime but the outer one soon overgrows the inner and passes over the endostome The outer integument is 2 4 layers of cells thick. In the micropylar region, its cells become progressively larger than the rest. The inner integument is 2 3 layers thick. It overgrows the tip of the nucellus by the time of formation of a 2-or 4-nucleate embryo sac (Figs. 19 20) The micropyle is formed of both the integuments with the exostome and endostome lying almost in a straight line

The hypodermal single-celled archesporium functions directly as megaspore mother cell (Fig 16) which before undergoing any division elongates considerably and almost reaches the base of the nucellus. It is only after reaching the base of the nucellus, that a megaspore mother cell is subjected to the normal meiotic mitosis resulting in the formation of a linear tetrad of megaspores (Fig 17) of which only the chalazal one is functional (Figs. 18 19

The functional megaspore enlarges, while the remaining three megaspores degenerate The nucleus of the functional megaspore divides to form a 2 nucleate embryo sac (Fig 19) whose nuclei move to opposite poles leaving a vacuole in the centre of the embryo sac Both the nuclei of the 2-nucleate embryo sac divide simultaneously resulting in the formation of a four nucleate embryo sac The micropylar end of the four nucleate embryo sac is broader while the chalazal end is narrower There is a prominent vacuole in the centre of the embryo sac (Fig 20) The enlarging four nucleate embryo sac dissolves the nucellar cells lying towards the micropylar end.

The next division of the embryo sac nuclei which is again simultaneous results in the formation of an eight-nucleate embryo sac (Figs. 21 a and b and 22) which thus shows the Polygonum type of development (Maheshwari

1950). The egg apparatus consists of three cells so also the antipodals. There are two polar nuclei. The antipodals are ephemeral. The major part of the embryo sac lies in direct contact with the inner integument.

Endosperm.—The primary endosperm nucleus is formed as a result of triple fusion. Its divisions give rise to a free nuclear endosperm (Figs. 23 24). It is only after several endosperm nuclei are formed that the egg undergoes division. As the division of endosperm progresses, a number of nuclei are accumulated at the micropylar end embedded in a dense mass of cytoplasm. When the zygote starts dividing these free endosperm nuclei are drawn back to the periphery forming a big vacuole at the centre. The laying down of walls between endosperm nuclei takes place quite late and globular pro-embryo is seen to lie in free nuclear endosperm (Fig. 24).

Embryo.—The zygote becomes much elongated. It then undergoes a transverse division producing a terminal cell *ca* and a basal cell *cb* (Fig. 25). The latter divides transversely giving rise to *m* and *ci* (Fig. 26). Next *m* undergoes a transverse division giving rise to *f* and *d* (Fig. 27). This is followed by the longitudinal division of the terminal cell *ca* producing two juxtaposed cells *q* (Figs. 27 28). By the elongation of *ci*, *f* *d* and further divisions of some of these, a long suspensor is produced which helps in pushing the growing embryo deep into the endosperm. The lowermost cell *h* of the suspensor probably functions as a hypophysis (Fig. 31).

The two juxtaposed cells from *ca*, already mentioned, undergo another longitudinal division at right angles to the previous plane to form a quadrant of 4 cells (Fig. 29). A transverse division of cells of quadrant *q* gives rise to an octant with the cells arranged in two tiers l and l' (Fig. 30). Periclinal walls are then laid down in each of the cells of the octant producing a small globular embryo (Fig. 31).

After the cells of the octant have completed their periclinal divisions, the hypophysis initial *h* undergoes a transverse division (Fig. 32). The upper of the two daughter cells divides longitudinally (Fig. 33) and slightly later the lower also divides in the same plane (Fig. 34). By further divisions, the derivatives of *h* give rise to the root cap and the tip of the radicle.

The globular embryo becomes heart shaped due to further divisions and development. It ultimately gives rise to a mature embryo consisting of two large cotyledons, a plumule and a hypocotyl.

Thus the embryo development follows the *Capsella* variation of Onagrad type (Johansen, 1950) with a slight variation that the terminal cell of the suspensor is neither vesicular nor swollen.

SUMMARY

(1) Microsporogenesis and development of male gametophyte proceed in the normal way. Shedding of pollen grains takes place at three-celled stage.
(2) Development of female gametophyte follows the Polygonum type.

(3) Endosperm development is of the Nuclear type.

(4) The development of embryo follows the *Capsella* variation of Onagrad type.

Acknowledgements

I am obliged to Professor K N Kaul, F L S Director National Botanic Gardens, for providing proper facilities and for encouragement, and to C. S. I R. for the award of a Research Fellowship. I am also indebted to Dr Bahadur Singh, Assistant Director National Botanic Gardens for suggesting this problem and giving his guidance.

References

1 Hooker J D 1875 Flora of British India." I

2 Johansen, D A. 1950 : "Plant Embryology Waltham, Mass., U S.A.

3 Maheshwari, P 1950 : "An Introduction to the Embryology of Angiosperms. *Mc. Graw-Hill New York.*

Explanation to Plates

Figs. 1 15 Microsporogenesis and development of male gametophyte

Fig 1 T S. anther showing a group of archesporial cell. x 850 Note—Only some have divided periclinally so far

Fig 2. T S same shows periclinal division of primary parietal layer. x 850

Fig 3 T S. same showing periclinal division of some cell of the third layer from above. x 850

Fig 4 T S. same showing epidermis, endothecium, degenerating middle layer and sporogenous cells. x 850

Fig 5 Endothecium with fibrous band and epidermis. x 850

Fig 6 Microspore mother cell synaptic knot of prophase I x 1250

Fig 7 Same during metaphase I x 1250

Fig 8 Same during anaphase I x 1250

Fig 9 Same during telophase II x 1250

Fig 10 Microspore mother cell after complete reduction division. x 1250

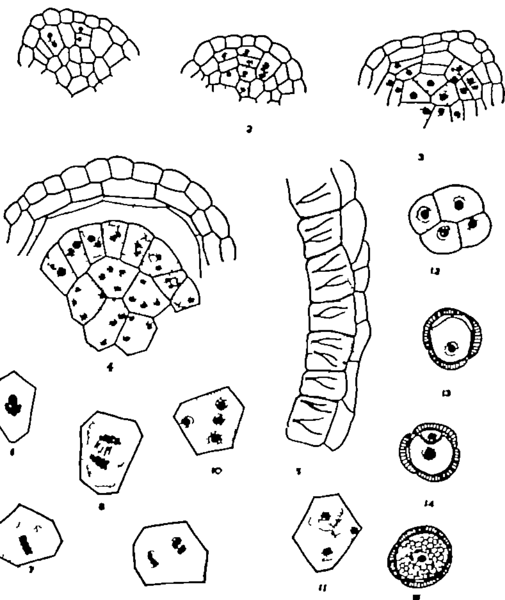
Fig 11 Tetrahedral arrangement of microspore tetrad. x 1250

Fig 12. Isobilateral arrangement of microspore tetrad. x 1250

Fig 13 Uninucleate pollen grain x 850

Fig 14 Bicelled pollen grain x 850

Fig 15 Tricelled pollen grain. x 1250


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Figs. 16-22 Megasporogenesis and development of female gametophyte. *Note* —Of the two integuments only the inner has been shown.

Fig 16 L S ovule showing megaspore mother cell and initiation of both the integuments x 850

Fig 17 L. S nucellus with inner integument to show the linear tetrad of megaspores. x 850

Fig 18. L. S same showing the upper three megaspores degenerating while the chalazal megaspore functions. x 850

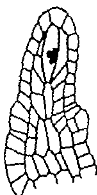
Fig 19 L. S. same shows a binucleate embryo sac capped with three degenerating megaspores. x 850

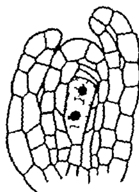
Fig 20 L. S same showing tetranucleate embryo sac. x 850

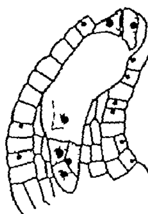
Fig 21-a. Embryo sac with two synergids, three antipodal cells and the chalazal polar nucleus. x 850

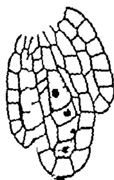
Fig 21 b Rest of the embryo sac of fig *21-a* with the egg and the micropylar polar nucleus. x 850

Fig 22. Reconstructed embryo sac from figs. *21-a* and *21-b* showing the egg apparatus, antipodals and polar nuclei. x 850

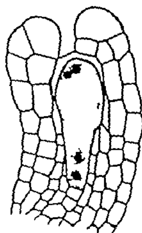

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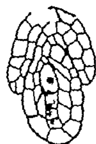

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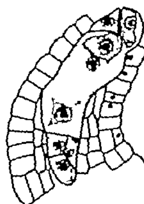

21


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21b


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Figs. 23-34. Development of endosperm and embryo.

Fig. 23. Embryo sac showing the zygote, degenerating synergids and free endosperm nuclei. x [illegible]

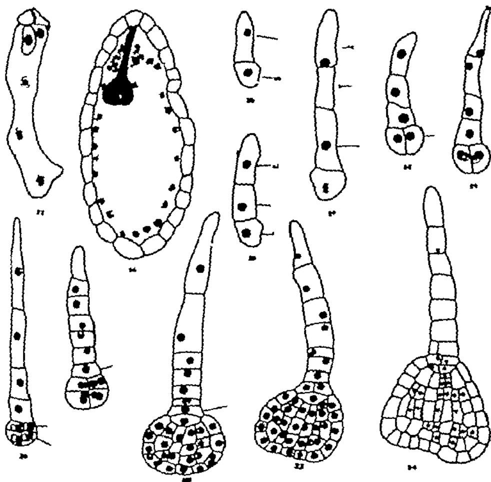
Fig. 24. Same more advanced; free nuclear endosperm and [illegible] of endosperm [illegible] x [illegible]

Fig. 25. Two-celled proembryo. x 565

Figs. 25-33. Different stages of embryo development. x 565

Fig. 34. A heart-shaped embryo. x 565

(h hypophysis)



Early yield of larger fruits thus obtained would bring much profit to the growers as early marketing and high production will fetch premium prices.

(iii) *Effect on fruit quality*

TABLE 5

*Effect of 2, 4 5-T on fruit quality of loquat**

Concentrations of 2,4,5-T in parts per million	Wt. of pulp per fruit in gm.	Precent incre ase in wt. of pulp per fruit over control	Wt. of seed per fruit in gms.	Ascorbic acid mgms/100 gms. of edible portion
5	9.91	18.68	3.52	3.97
10	10.69	28.02	3.35	4.20
25	14.30	71.25	3.30	4.61
50	15.14	81.31	3.14	4.76
Control	8.35	——	5.67	3.01

* Average of ten fruits.

Weight of pulp per fruit was increased with increase in concentration of 2, 4, 5-T spray. A significant variation was found in yield of pulp under 25 and 50 p. p. m. sprays which exceeded the control by 71.25 and 81.31 per cent respectively.

2, 4 5-T was found effective at all concentrations in reducing the number of seeds and seed weight per fruit in comparison to fruits from control bunches.

Chemical analysis of marketable fruits under different treatments of 2,4 5 T showed increase in the ascorbic acid content of the fruits in comparison to control.

On the basis of the results described here, it is clear that blossom sprays of 2,4,5-T may be used with advantage in increasing fruit-set, fruit-size quality yield and also to hasten the ripening of loquat fruits.

SUMMARY

The blossoms of loquat Variety Golden Yellow were sprayed with 5 10 25 and 50 p. p. m. of 2 4 5-T and the following results were obtained —

1. 5 and 10 p. p. m. increased fruit-set over control.

2. 50 p. p. m. of 2, 4 5-T sprays hastened the maturity of the fruits by 10 days.

3. Considerable increase in fruit-size mean fruit weight and pulp weight per fruit over control was found in 25 and 50 p. p. m.

4. An increase in ascorbic acid content was found in treated fruits.

Literature Cited

1 Association of Vitamin Chemists. 1951 Methods of Vitamin Assay

2 Anonymous 1958 Horticulture Divisio D S I R. Effect of hormones on size and maturity of apricots and plums Orchard N Z. 31 319

3 Blondeau R. & Crane J C. 1948 Early maturation of calimyrna fig fruits by means of synthetic hormone sprays. *Science* 108 : 719-20.

4 Crane J C. & Blondeau, R. 1949: Controlled growth of fig fruits by synthetic hormone application. *Proc Amer Soc hort. Sci* 54 10.

5 Crane J C. & R M. Brooks. 1952: Growth of apricot fruits as influenced by 2 4 5-T application *Ibid* 59 : 218-224.

6 Crane J C. 1953 Preharvest drop, size maturity of apricots as affected by 4 5-T *Proc Amer Soc hort Sci* 53 : 73.

7 Crane J C. 195_ Fruit growth of 4 fig vars. as measured by diameter and fresh weight. *Proc Amer Soc hort. Sci.* 59 p. 221

8 Dexter J F., Griggs W.H & Crane J C. 1950 The effect of growth regulating chemicals applied during the bloom period on the subsequent set of Bartlett pears. *Proc Amer Soc hort Sci.* 55 p. 137

9 Farmer A. J.E. Hawkins & G E. Robert. 19_3 2 4 5-T hasten maturity of afira and Moorpark apricots. Orchard N Z 31 75-9

10 Gustafson F G 1936 Inducement of fruit development by growth regulating chemicals. *Proc Nat L A Sci.* 22 628-636 1936.

11 Hatton, T T 195_ Responses of the Delicious apple to sprays of 4 5-Trichlorophenoxy acetic acid. *Proc. Amer Soc hort. Sci* 63 59-62.

12 Hegdon R. J 1955: The effect of _ 4 5-T on development and ripening of 18 ars of peach. *Proc Amer Sci. hort. S L* 58 p. 73-79

13 Marth, P C., Harley C. P & Haris, A L. 19_0 Effect of 2 4 5-T on ripening of apple and peach. *Science* 111 : 331 3_

14 Randhawa G S. & Dhuria H. S. 19 9 Effect of Gibrellic Acid 4 5-T 2 4-D on fruit-set, drop size & total yield in sweet lime *I Jan J Hort.* 16, p. 206.

15 Randhawa, G S Singh J P & Khanna S. S 19_ Effect of gibrellic acid and some other growth regulators on fruit-set size total yield and quality in Phalsa *I Jan J Hort.* 16 p. 202.

16 Singh, J P Randhawa G.S & Rajput, C B S 19_0: Effect of Plant Regulators on fruit development, ripening drop yield size and quality of loquat Eriobotrya japonica, var Improved Golden Yellow & Pale Yellow. *Ind J Hort* 17 : 156-161

17 Zielinski, Q B Marth, P C. & Price V. E. 1957: Effect of 2 4 5-T on maturity of prunes. *Proc Amer Soc hort Sci* 58 6_-63.

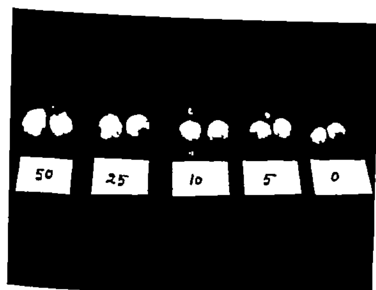


Fig. 1. showing the size of loquat fruits obtained under the influence of different concentrations of 2 4 5-T

Literature Cited

1 Anon ... Vitamin ... 19 ... M ...
Anony ... 19 ... Di ... sion ... hormones on
... rity ... cots and plums Och ... 31 319
3 Blondea ... & Crane J C 1948 Early maturation ... of ...
... yn hetic hormone sprays. Science 108 ...
4 Cr ... J C & Bl nd ... R 1949 Controlled growth of ... by ...
appl ... at ... Amer Soc Hort Sci 54 10
5 Cra ... J C & P M ... 195 Growth of ... fruit as ... by ...
... plication Ibid 59 ... 10-2 ...
6 C ... J C 19 ... d ... mat ... lty of apricot ... d by ... T
P ... Amer S ... Hort S 55 ... 75
7 Cra ... J C ... fruit growth of ... measured by ...
... Proc Amer S ... Hort S 59 p 231
8 Dera ... J P G ... W H ... Cra ... J C 1950 The effect of ...
... period ...
Bartlett pears Proc Amer S ... Hort Sci 55 ... 137
9 F ... J T H ... & O ... Robert ... 4 ...
... Moorpark ... in Orland N Z 51 ...
10 ... 1 G 193 Inducement of fruit development by ...
Proc ... 6 0-636 193
11 H ... T T 1955 Responses of the Delicious apple to ... of 2 4 5 T ...
... Amer ... Hort Sci 6 ... 0 6...
12 H ... R J 1955 The effect of ... 4 5-T on develop ...
of peach Proc Amer ... Hort S 58 p 3...
13 M ... P C ... Hartly C P & H ... 19 0 Eff ... 1 ...
... peach Science 111 ... 331
14 R ... G S & D ... H S 19 9 Eff ... of Gibb ...
... total yield ... J ... 16
p 27
15 R ... G S Singh J P & ... S S 19 ... Effect ...
growth regulators on fruit size ...
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STUDIES ON THE VELOCITY OF FLOW OF LIQUIDS THROUGH ADSORBENT COLUMNS

Part XII Possible Applications of the Phenomenon

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The velocity of flow of liquids through porous media is a phenomenon of great importance in irrigation, drainage, petroleum wells, agriculture and a number of other important industrial problems. In any packed column made of suitable adsorbents like silica, alumina, fuller's earth etc., there are a large number of voids which form the pores through which the liquids can flow or permeate with a certain velocity. These pores are arranged in such a way as to form an inter-connected network of channels which allow the liquids to form the stream of flow. The earlier workers [1-4] have studied this problem mainly in the aspect of porosity, pore spaces, geometrical shapes of particles and hydrostatic pressure and a number of mathematical equations based on these factors have been given. But there is hardly any mention in the literature that the rate of flow of liquids also depends upon the surface active forces of the particles forming the columns and the structural characteristics and the functional groups of the flowing liquids. In order to bring out the evidences of the role of molecular structure on the velocity of flow of liquids through porous columns, extensive investigations [9] under controlled conditions of the height of the column, pressure, temperature, size and shape of the particles have been carried out and an empirical equation $\frac{Vh}{t} \cdot \frac{\eta}{d} = K$, for the flow of liquids through adsorbent columns has been derived [8]. In the present communication, the author has discussed some possible applications of the flow of liquids through porous media.

Interesting references of the velocity of solvents through the adsorbent columns are found in the development of chromatography. LeRosen [5] evolved a method for the standardisation of adsorbents by determining the relative rates of the movement of a zone (mm/min.) and the rate of flow (mm/min.) of the developer. The ratio of the rate of movement of the zone and the rate of flow of the developer Vc, when a constant flow has been reached, gave a certain value which was designated as R. The approximate strength of the adsorption affinity has been characterised by the value of R. LeRosen R is equivalent to R of Martin and Synge. Hence the value of the velocity of flow of solvents through adsorbent columns finds its use in chromatography in which its significance has proved to be of a far reaching character.

The elution of colouring matters in paper partition chromatography depends upon the relative movements of the adsorbed zone and the eluant. Jacques and Mathieus [6] observed that the eluting power of the solvents was proportional to their dielectric constants and the movement of the zone was due to the adsorption of the eluant and displacement of the zone by the solvent Eluants have thus been standardised by studing the relative rate of movements of the eluting solvent and the adsorbed zone

The different homologous series of organic compounds give a characteristic value for the flow constant K in the equation $\frac{Vh}{t} \quad \frac{\eta}{d} = K$ [7] These flow constants determined under standard conditions of pressure temperature size grade and packing may enable us to identify the family to which a particular liquid belongs.

The flow of a liquid through an adsorbent column mainly depends upon the surface active forces of the adsorbent and the structural characteristics of the flowing liquid The mixtures of different liquids may therefore be suitably fractionated (cf. distillation) by flowing them through different adsorbent column as under standard conditions the constituents of mixtures of liquids will have different rates of flow

Studies on the velocity of flow may be put to rigorous tests in characterising the geological sediments and stratified rocks Geologists deal with intricate problems on the internal structures of the earth s crust and flow of water oil and gases through the pores and channels of the underground layers. The sedimentary rocks vary in their composition according to the r origin which are classifed into the groups of (i) clasti (ii) organic and (iii) chem al dep sits. The clastic deposits comprise the gravels sand silt and clay The organic dep s ts are composed f the decomp ed re dues f plants and a imal r ma ns wh ch ultim tely turn into th sources of petr leum wl ile th chemical deposit are ma nly the precipitat d minerals s h a silica alum na ron and otl r mineral ses mixed up with solul e salts. From the ompos tion of these sed ments it s evident that th y posses pecifi [illegible] h mical p operti es an l [illegible] ltera t ristics by vi t [illegible] of s l ch th velocity of fl w w ll be affect d un ler con l tions f pr per [illegible] Th [illegible] t [illegible] f pa tic ilar l qu l through th sampl c lum [illegible] n l h [illegible] d mentary r ks may form [illegible] t l [illegible] E per men ts based [illegible] apprai [illegible] t [illegible] pp [illegible] c nd t ons of p k ng in a tu It may l [illegible] t [illegible] keep the grad n of [illegible] d pa k th c mn rt fically [illegible] m anst mak th p k g [illegible] un f rm [illegible] Th velo of fl w of l [illegible] l dete m [illegible] keep [illegible] the temperat [illegible] and pre [illegible] tant m st [illegible] f th constant K n $\frac{Vh}{t}$ [illegible] d [illegible] K, f r [illegible] rent sed ments c [illegible] teri ti of th tructure f th [illegible] and the r [illegible]

chemical properties. If experiments are conducted on the flow characteristics of a liquid through the sedimentary rocks, the geologists may discover one more suitable index to supplement the existing ones for spotting out the organic deposits and petroleum beds, wherever they exist.

In the light of the prevailing view of the soil physicists, water movement in soil takes place through the capillaries of the small pores as well as through the passages of larger ones. These movements are brought about by the action of gravity or capillary pull, either alone, or in combination according to the size of the pores of the system. But the movement or transmission of liquids through the porous columns elicits further that the flow of a liquid also depends on a new variable which finds justification in the surface properties of the material of the column and the structure of molecules. This observation leads to the agreeable suggestion that any characteristic physico-chemical change brought about in the soil may be indicated by the variations in the velocity of flow of a liquid. Thus liquid if used as a reference, comparative data under identical conditions may be obtained for the variations of the soil properties.

Soil is a dynamic body of composite nature which has been changing its character very slowly and continuously according to the geographical and climatic conditions. The soils are usually characterised by their (i) clay minerals, (ii) clay content, (iii) organic matter (iv) mineral salts, (v) soil structure (vi) exchangeable bases and (vii) size distribution of the particles. Since there are so many variables, it is difficult to determine which of them have suffered a change due to weathering hydration, or chemical decomposition, and to what extent these changes have been brought about. Nonetheless, the velocity of flow will vary depending upon the variations in one, two or all the conditioning factors which characterise the soil. Thus it follows that if a reference liquid, say benzene, is made to flow under identically controlled conditions through a column of the soil, the velocity of transmission or the constants calculated from the equation $\frac{Vh}{t} \cdot \frac{\eta}{d} = K$ will serve as an index for the variations in the structure of the soil brought about by physical and chemical changes. The determination of the velocity of flow of liquids may also prove a very significant aspect of study in soil stabilisation for road making.

It may be quite interesting to visualise the significance of K in $\frac{Vh}{t} \cdot \frac{\eta}{d} = K$, as a structural constant like parachor or rheochor. The density (d) of the liquid being in the denominator suggests, ceteris paribus, that K is the specific constant for the flow of a family of organic liquids. Hence, if values of K for alcohols, esters or ketones are multiplied by the molecular weights of the ascending homologoues, the products should give a constant difference due to the addition of a CH group from member to member. This has been actually observed [5] but one must be cautioned to declare a fixed value for these constants as well as for the differences of the products, because there is method of checking up whether arrangement of particles regular

size and shape of the pores are identical or not If it were so there would have been a possibility of emergence of a new physical constant (fluidochor ?) for the liquids from their velocity of flow through the adsorbent columns

Summary

The problem of the flow of liquids through porous media has been discussed with regard to its possible applications in chromatographic processes, identification of liquids belonging to different families, fractionation of the mixtures of liquids, characterisation of various geological and sedimentary rocks with a view to spotting out organic deposits and petroleum beds, and the characterisation of various types of soils in soil engineering and road making A possibility of emergence of a new physical constant (fluidochor ?) for the liquids from their velocity of flow through the adsorbent columns has also been pointed out

References

[1]. *Darcy H : "Les fontaines publiques de la ville de Dijon* 1856.
[2] Slichter C S U.S. Survey 19th Annual Report Part II 293.
[3] Fancher G.H Lewis, J A. and Barnes, K. B. : *Min. Ind. Exp Stn. Penn. State* College Bull 12 1933.
[4] Zunker F : *Gh Commn I Intn Soc Soil Sci.* (Groningen) 1933, *B* 18.
[5] LeRosen : *J Amer Chem. Soc* 1912, 64 1905; *Anal Chem.* 1947 19 189
[6]. Jacques, J and Mathieu: *Bull. Soc Chem* France 1946, 94
[7] Gupta, D.R and Bhattacharya Abani K. : *J Indian Chem. Soc* 19 3 30 0 1; 1954 31 431
[8] Gupta, D.R. and Bhattacharya, Abani K. *J. Phys Chem* 1957 59 700
[9] Gupta, D.R. : Thesis, Agra University 1950.

NUTRITIONAL REQUIREMENTS OF *STREPTOMYCES GRISEUS* AGRA STRAIN ON THE PRODUCTION OF A FUNGISTATIC SUBSTANCE. III pH OF THE MEDIUM

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Introduction

Streptomyces griseus Agra strain produces a fungistatic substance active against *Alternaria solani* and a few other fungi and bacteria (Basu Chaudhary 1959). The fungistatic substance is different from those produced by other strains of *Streptomyces griseus*. The effect of different carbon and nitrogen sources on the production of the fungistatic substance has been studied by the author (1961 1962)

The metabolism of active substances produced by actinomycetes and other organism has been found to be greatly influenced by the pH of the medium. The effect of pH on the production of penicillin has been studied by Raper & Alexender (1945) Foster *et al* (1946) Johnson (1946) Moyer & Coghill (1947), Stefaniak *et al* (1946) Stone & Farrell (1946) Moyer & Coghill (1946) found that the loss of penicillin may be substantial outside the pH range of 5-7.5 Jarvis & Johnson (1947) found that in lactose-glucose medium containing both ammonium acetate and lactate, the optimal pH for penicillin production was 7.3 Gottlieb & Anderson (1947) reported that synthesis of streptomycin proceeded in the presence of oxygen and there was no correlation between pH changes and the streptomycin production. Garner *et al.* (1953) observed that initial pH above 8.0 was detrimental to yields although good growth was obtained and there appears to be little correlation with the eventual yield over the initial pH range 5-8. Brian and his associates have studied the effect of pH on the production of gliotoxin (1945), glutinosin (1947) gladiolic acid (1948) alternaric acid (1951) canescin (1953) and others.

This investigation deals with the effect of different initial pH of the medium on the growth of *Streptomyces griseus* Agra strain and the production of the fungistatic substance.

Method and Material

Streptomyces griseus Agra strain was cultured in a medium composed of glucose 10 gm., sodium chloride 5 gm. meat extract 5 gm. water 1000 c.c. 30 c.c. of the medium was dispensed in flat bottles and sterilized at 12 lbs. pressure for half an hour The different pH ranges were adjusted with HCl and KOH solutions prior to autoclaving. The bottles were inoculated

with twelve day old culture and incubated at 25°C. Five replicates of each treatment were maintained The fungistatic activity was measured by the serial dilution method (Basu Chaudhary 1959) at intervals of 7 10 13 and 16 days after which the final pH of the medium and the dry-weight of the mycelium were taken

RESULT

Table showing the effect of different initial pH on the production of the active substance in SD units by S griseus Agra strain and its growth

(Mean of five replicates)

Initial pH	D 7	A 10	Y 13	S 16	Final pH	Dry-weight in gm
3 4	NO GROWTH					
5 4	VERY SPARSE GROWTH					
6 6	8 0	14.0	32 0	64 0	5.5	1 0173
7 0	32 0	64 0	128 0	256 0	7 4	1.9755
8 2	32 0	44 0	89 6	153 6	11.7	1.8952
9 0	16 0	32.0	51.2	102.4	9.5	1.6019

The data presented in the table reveal that the organism is unable to grow in high acidic pH range In 3 4 there was no growth of the organism whereas very sparse growth took place in pH 5 4 The growth in general increases when the initial pH of the medium was raised The mean mycelial dry weights were 1 0173 gm., 1 9755 gm., 1.8952 gm., 1 6019 gm at pH 6.6 7 0 8.2 and 9 0 respectively the optimum being at 7 0 The production of the fungistatic substance also follows the same trend There is a direct correlation between the growth of the organism and the production of the fungistatic substance

DISCUSSION

The result obtained in this investigation is in line with the general observation that alkalies and alkali substrates favour the growth of actinomycetes whereas acid and acidic substrates have a retarding effect (Waksman 19?? The growth of the organism was almost nil in pH levels 3 4 and 5 4 also the fungistatic activity was correspondingly nil. As the pH was raised both the growth and the production of the fungistatic substance increased The optimal results were obtained at 7 0 However the result of the present investigation is contrary to the observation made by Gottlieb & Anderson (19?) and Garner et al (19?3) in that the pH influences the production of the fungistatic substance

SUMMARY

The effect of different initial pH ranges (3 4 5 4 6.6 7 0 8.2 and 9 0) on the growth of *Streptomyces griseus* Agra strain and the production of a fungistatic substance has been investigated It has been found that the growth of the organism was almost nil in pH levels 3 4 and 5 4 also the fungistatic activity was correspondingly nil. As the pH was raised both the growth and the production of the fungistatic substance increased, optimum being at 7 0 Growth and the production of the active substance were closely related to initial pH of the medium.

ACKNOWLEDGEMENTS

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REFERENCES

1 Basu Chaudhary K. C. 1959 Studies on the production of fungistatic substance by certain soil fungi. *Ph. D Thesis Agra University Agra.*

2. Basu Chaudhary K. C. 1961 ; Nutritional requirements of *Streptomyces griseus* Agra strain on the production of fungistatic substance. I Carbon sources *Proc. Nat Acad. Sc.* (India) B 31 268-272.

3. Basu Chaudhary K. C. 1962 Nutritional requirements of *Streptomyces griseus* Agra strain on the production of a fungistatic substance. II Nitrogen sources. *Agra Univ. J Res* (Sci.) XI 183-186.

4. Brian, P W & Hemming 1945 Gliotoxin fungistatic metabolic product of *Trichoderma viride*. *Ann appl. Biol* 32 214-220

5. Brian, P W Curtis, P J & Hemming, H.G. 1947 Glutinosin fungistatic metabolic product of the mould *Metarrhizium glutinosum* S Pope. *Proc. roy Soc.* B 135 106-132

6 Brian, P W Curtis, P J & Hemming H G 1948 Gladiolic acid an antibiotic substance produced by *Penicillium gladioli* McCull & Thom. *J gen. Microbiol* 2 341 355.

7 Brian P W Curtis, P J Hemming, H G. Jefferys E. G Unwin, C. H. & Wright, J M. 1951 Alternaric acid biologically active metabolic product of *Alternaria solani* (Ell. & Mart.) Jones & Grout its production isolation and antifungal properties *Biol* 5 619-632

8. Brian P W Hemming H G Moffat, J S & Unwin C. H. 1953 Canescin an antibiotic produced by *Penicillium canescens* *Trans. Brit. mycol Soc.* 36 243-247

9 Foster J W Woodruff H. B. & M Daniel L.E. 1946 Microbiological aspects of penicillin. IV Production of penicillin in submerged cultures of *Penicillium notatum* *J Bact.* 51 465-478.

10. Garner H. R. Koffler H. Tetrault P A. Fahmy M. Mallet M V Frost R A., Phillips, R. L. & Bohonos N 1953 Factors affecting streptomycin yields. *Amer J Bot* 40 289-296

11 Gottlieb D & Anderson, H W 1947 Morphological and physiological factors in streptomycin production. *Bull Torrey Bot. Club* 74 293-307

12. Jarvis, F G. & Johnson, M. J 1947 The role of the constituents of synthetic media for penicillin production. *J amer Chem. Soc* 69 3010

with twelve day old culture and incubated at 25°C Five replicates of e. treatment were maintained. The fungistatic activity was measured by serial dilution method (Basu Chaudhary 1959) at intervals of 7 10 13 and days after which the final pH of the medium and the dry-weight of mycelium were taken.

RESULT

Table showing the effect of different initial pH on the production of the actinomycetes in SD units by S griseus Agra strain and its growth

(Mean of five replicates)

Initial pH	D	A	Y	S	Final pH	Dry-weight in gm.
	7	10	13	16		
3 4	NO GROWTH					
5 4	VERY SPARSE GROWTH					
6 6	8 0	14.0	32 0	64 0	5.5	1 0173
7 0	32 0	64 0	128 0	256 0	7 4	1.9 55
8 2	32.0	44.8	89 6	153 6	8.7	1.895.
9 0	16 0	32.0	51.2	102.4	9.5	1.0919

The data presented in the table reveal that the organism is unable to grow in high acidic pH range In 3.4 there was no growth of the organism whereas very sparse growth took place in pH 5 4 The growth in gen. increases when the initial pH of the medium was raised The mean myce dry weights were 1.0173 gm., 1 9755 gm 1.8957 gm. 1.0919 gm., at pH 6 7 0, 8.2 and 9 0 respectively the optimum being at 7 0 The production of fungistatic substance also follows the same trend. There is a direct correlat between the growth of the organism and the production of the fungis substance.

DISCUSSION

The result obtained in this investigation is in line with the pre observation that alkalies and alkali substrates favour the growth of actinomy tes whereas acids and acidic substrates have a retarding effect (Waksman 19 The growth of the organism was almost nil in pH levels 3 4 and 5.4 the fungistatic activity was correspondingly nil. As the pH was raised the growth and the production of the fungistatic substance increased optimal result was obtained at 7 0 However the result of the pre investigation is contrary to the observations made by Gottl & A (1947) and Garner et al (1953) in that the pH influences production of fungistatic substance.

ON A NEW SPECIES OF *PARAMONOSTOMUM* LÜHE, 1909 (TREMATODA NOTOCOTYLIDAE) FROM *ANAS CRECCA* L.

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This genus, in Indian birds has been studied by Moghe (1932) Lal (1936) and Baugh (1958) Moghe described the species *P microstomum* from the posterior part of the small intestine of *Philomachus pugnax* Lal (1936) after giving an account of his two species *P casarcum* and *P querquedulae* collected from *Casarca rutila* and *Querquedula circia* respectively removed Moghe s form to his new genus *Neoparamonostomum* since suppressed as a synonym of *Paramonostomum* (Harwood, 1939) Baugh (1958) described two other species, *P fulicai* from caeca of *Fulica atra* and *P nettiona* from rectum of *Nettion crecca* and also appended a key for the five Indian species.

The specimens described below were available from a wild duck *Anas crecca** examined during the teaching season 1960-61 Of the six specimens collected from the caeca, one was serially cut and stained. This form had previously been encountered during the examination of an unidentified species of wild ducks by one of us (B P P) and the stained preparations of this material were also available for study

Paramonostomum harwoodi n sp

All specimens sexually mature were grey in colour and of an elongate form with bluntly rounded ends and with the concave ventral surface having curved margins. The mounted specimens (Fig. 1) measure 2 04—2 33 in length and 0 70—0 81 in maximum width attained at about middle of the body There are no ventral glands but on the ventral surface spines are present. The oral sucker terminal and well-developed measures 0 083 × 0 10 in size. The mouth opens into a short oesophagus, 0.133 long, leading into the intestinal caeca which exhibit small lateral diverticulae along their course and terminate at a distance of 0 083-0 183 on right and 0 11-0 166 on the left sid from th posterior end of the body

The subterminal excretory pore lies at a distance of 0 25 from the posterior end. The two lobed testes, symmetrically arranged, lie extracaecally in the posterior part of the body The right testis measures 0 336-0 430 × 0 166-0 183 and left one 0 33-0 42 × 0 15-0 183 in size. The well developed and convoluted external seminal vesicle extends from the level of

*Identified from "The Book of Indian Birds" by Salim Ali, (1955).

the anterior margin of the vitellaria to the base of the elongated flask-shaped cirrus sac which, medially situated and measuring 0 533—0 683 in length and 0 083 in breadth, has its basal part occupied by the coiled internal seminal vesicle. The pars-prostatica, thickly surrounded by prostatic cells, leads into the ductus ejaculatorius which opens into the genital pore situated immediately behind the oral sucker

The lobed intercaecal ovary lies in the middle of the testicular zone and measures 0 166-0 22×0 166 in size. The well-developed vitellaria are extracaecal and extend from near the anterior border of the testes to near the middle of the body length. The transverse vitelline ducts, from the two sides, pass inwards from near the posterior limits of vitellaria and join to form the reservoir on the anterior level of the shell-gland mass which, situated just in front of the ovary measures 0 15×0 15 in size. The uterus completely intercaecal throughout its extent has 18-20 transverse coils of which the larger number in the vitelline zone continue into the 3-4 previtelline coils. The transverse coils do not extend to the base of the cirrus sac but in the area occupied by the external seminal vesicle the uterus runs as a thinner but straight tube finally leading into the thick walled metraterm of the same length as the cirrus sac and running along its side terminates at the common genital pore The eggs are small, provided with a filament at each pole and excluding the filament measure 18.5×11 25 μ in size.

HOST *Anas crecca*
HABITAT Caecum
LOCALITY Mathura.
Type specimen deposited in the Department of Parasitology U P College of Veterinary Science & Animal Husbandry Mathura

DISCUSSION

Amongst the fourteen species assigned to this genus viz *P* [illegible] (Mehlis in Creplin 1846) Lühe 1909 *P brantae* Bullock 19[illegible] *P* [illegible] Yamaguti, 1935 *P catarium* Lal 1936 *P elongatum* Yamaguti 1931 *P* [illegible] Baugh 19[illegible]8 *P* [illegible] Travassos 1921 *P* [illegible] Ku 19[illegible] *P* [illegible] *stomum* Mogle 1937 *P nettioni* Baugh 1948 *P* [illegible] Caballero 19[illegible] *P ovatum* Hsu 193[illegible] *P parvum* Stunkard et Dunihue 1931 and *P* [illegible] Lal, 1936 so far known from birds the present specimens on account of the more forward position of the genital pore come closer to the species *P* [illegible] Lal 1936 *P* [illegible] Lal 19[illegible] and *P nett*[illegible] Baugh 1[illegible] *P* [illegible] and *P nettioni* are both distinct from it on account of the intestinal caeca being simple and devoid of diverticulae though the genital pore lies [illegible] of the caudal margin of the oral sucker The present form [illegible] the character of the intestinal caeca with *P* [illegible] and [illegible] guished on account of the position of its genital pore which in *P* [illegible] lies near middle of oesophagus According [illegible] the specimens are a [illegible] new species *P* [illegible] in honour of Dr [illegible] D Hare [illegible]



(All measurements in mm.)

Fig 1 Ventral view of *Paramonostomum harwoodi* n.sp

Thanks are due to Sri C. V G Choudary Principal for the facilities provided in this work which forms a part of the Thesis submitted by D Nath and approved for the award of M V Sc. degree of Agra University and to Dr M. B Lal Head of the Zoology Department of Lucknow University for the loan of some literature.

REFERENCES

1 Baugh, S. C. (1958) Contributions to our knowledge of digenetic trematodes. III. *Proc. Nat. Acad Sc. India.* 28 (3) 205-225.

2 Bullock W L (1952) Two new species of monostomes from the Canada goose with review of *Paramonostomum alveatum* (Mehlis in Creplin, 1846) *J Par* 38 (5) 371 378.

3 Harwood P D (1939) Notes on Tennessee helminths. IV North American Trematodes of the subfamily Notocotylinae *J Tenn. Acad. Sc.* 14 421-437

4 Hsü, Y C. (1935) Trematodes of fowls in Soochow *Peking Nat Hist. Bull* 10 (2) 141 150

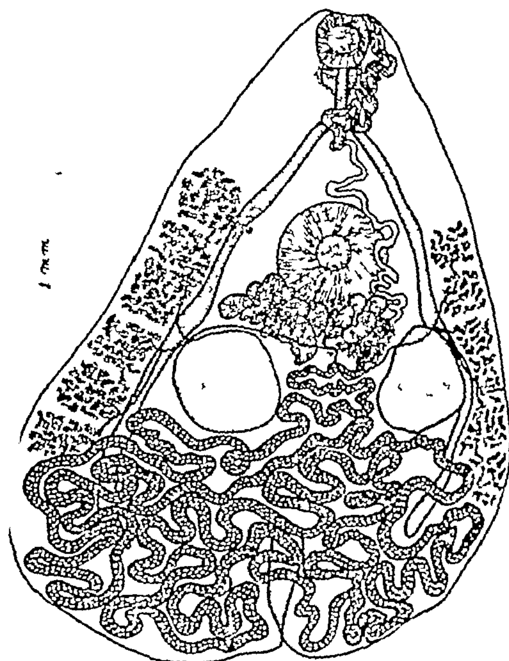
5 Ku, C. T (1938) New trematodes from Chinese birds *Peking Nat. Hist. Bull.* 13 (2) 129-136.

6 Lal M B. (1936) A review of the genus *Paramonostomum* Lühe with descriptions of tw new species and remarks on the genera of the subfamily Notocotylinae. *Proc. Ind. Acad Sc* 3 25-33

7 Moghe, M. A (1932) Tw new species of trematodes from Indian ruff (*Philomachus pugnax* Gray *Parasit.* 24 (1) 54-59

8 Stunkard H W & Dunihue, F W (1931) Notes on trematodes from Long Island duck lth description of new species. *Biol. Bull* [illegible] 179-186

the broader posterior extremity with a slight notch measures 3 857 in length. The maximum width 2 714 is attained in the post testicular region The cuticle all over has backwardly pointed spines of about 0 0? length which however



(All measurements are in mm)

Fig. 1 Ventral view of *Pr* [illegible]

gradually diminish posteriorly The well-developed oral sucker nearly terminal, is 0 299 × 0 3344 in size. The mouth opens directly into a prominent pharynx, which has an oval form and measures 0 173 × 0 176 in size with a prominent mass of unicellular gland cells around it. A short oesophagus, 0 176 long, bifurcates into the intestinal caeca extending posteriorly but ending at a distance of 0 661 on right and 0 704 on left sides from the posterior extremity The large ventral sucker nearly circular in outline and twice the size of the oral sucker 0 633 in diameter lies at a distance of 1 09 from the anterior end. The median excretory pore lying in the notch at the posterior end leads into the "Y" shaped excretory bladder

The two testes, symmetrically arranged lie intercaecally in the posterior half of the body with the right one, measuring 0 633 × 0 616 and somewhat of an oval outline while the left one irregularly oval in shape, is 0 737 × 0 500 in size. The long tubular but sinuous and coiled cirrus sac, situated to the left of the oesophagus extends posteriorly a little behind the intestinal bifurcation and anteriorly opens at the common genital pore situated close to the anterior margin of the oral sucker on its left side.

The ovary with about 16 lobes in all is median but transversely elongated. It lies close to the posterior border of the ventral sucker slightly overlapping it, and measures 0 492 × 0 914 in size. A diffuse shell-gland area lies posterodorsal to the ovary The vitellaria lateral and extracaecal, consist of seven groups of acini on the right side occupying a zone 1 9 of the total body length. The follicles on the left side however consist of six bunches distributed over a distance of 1 8 Anteriorly the follicles extend slightly beyond the level of acetabulum and posteriorly beyond the posterior limits of the testes reaching nearly the middle of the post-testicular area. The transverse vitelline ducts from the two sides pass inward to enter the shell gland mass situated just behind the acetabulum between the ovary and the anterior testis. The uterus, with its descending and ascending coils running a sinuous course, occupies the area between and behind the testes and finally the ascending limb passes towards the common genital pore along side of the cirrus sac to open through a metraterm also of slightly sinuous character into the common genital pore The uterus is filled with numerous eggs brown in colour and measuring 21 × 10 5 µ in size (in mounted specimen)

Subsequent to the discovery of the specimen in the oviduct of the domestic fowl and at times inside eggs, *Prosthogonimus* has been studied in a number of countries. Skrjabin and Massino (1925) as stated by Wittenberg et Eckmann had split up this genus into three subgenera, *Prosthogonimus Ultragenaltrema* and *Macrogenaltrema* Yamaguti (1958) on the other hand without recognising the subgenera included it under Prosthogonimidae Nicoll 1924

In the matter of speciation, amongst the large number of [illegible] so far proposed disagreement exists amongst the various workers Macy (1934)

in a detailed and useful monograph recognised in all sixteen species as valid. Witenberg and Eckman (1939) in their critical review regarded only seven of the twenty-three earlier known species as valid and these were *P. cuneatus* Nicoll 1915 *P. ovatus* (Rudolphi 1803) *P. dogieli* Skrjabin, 1916 *P. cuneatus* (Rudolphi 1809) *P. putschkowskii* *P. rudolphi* Skrjabin 1919 and *P. pellucidus*. Even in the list of these seven species, the validity of *P. putschkowskii*, *P. rudolphi* *P. cuneatus* and *P. dogieli* was doubted and some of the species cited as synonyms were inserted as "species inquirendae" without any mention of *P. indicus*. The characters relied upon by the joint authors in differentiating the species of *Prosthogonimus* included (1) the relative size of the oral and ventral suckers (2) the character of uterine coils (3) the shape and distribution of the vitellaria and the other characters used by most of the workers were thought to be of no real value due to age state of contraction or individual variation.

In his study of this genus Chauhan (1940) appears to have mostly followed Macy in accepting all the thirteen species under it but adding four other forms he excluded the three species, *P. skrjabini* *P. karausiaki* and *P. kurochi*, the first and second having been considered as synonyms of *P. anatinus* and the third of *P. putschkowskii*. *P. longusmorbificans* regarded by Macy (1934) as "species inquirendae" was included amongst the species alongwith *P. lini* Hsu 1935 *P. indicus* and *P. macroacetabulus*. Evidently Chauhan had not consulted the important publication of Witenberg et Eckman and his species *P. macroacetabulus* on scrutiny appears to be identical with *P. putschkowskii*. Besides *P. skrjabini* and *P. karausiaki* believed by Chauhan as synonyms of *P. anatinus* had been reduced as synonyms of *P. rudolphi*

Bhalerao et Gideon (1941) indicating that Witenberg et Eckman did not consider *P. indicus* followed their plan and regarded *P. indicus* a synonym of *P. putschkowskii*. In dealing with the then known representative species of *Prosthogonimus* recovered from Indian birds the joint authors did not comment on Chauhan's work.

The other species described from elsewhere subsequent to Chauhan's publication include *P. sinensis* Ku 1940 *P. fosus* Ku 1940 *P. [illegible]* Tubangui et Masilungan 1941 and *P. longusiformis* Yamaguti et Iwata 194[illegible]

Dollfus (1948) appears to have followed Macy (1934) and Chauhan (1940) in the key appended for the various species recognised under *Prosthogonimus* while Chen (1951) believed that all the twenty-seven species could be reduced to six species *P. cuneatus* *P. pellucidus* *P. rudolphi* *P. [illegible]* (known from China) *P. ovatus* and *P. dogieli* for which a key was given and *P. [illegible]* and *P. karausiaki* listed as "species inquirendae". The views expressed by Witenberg and Eckman essentially appear to have been accepted by Chen (1951) with the difference that *P. ovatus* included earlier [illegible] inquirendae" by Witenberg et Eckman and also suppressed as a synonym [illegible]

P rudolphi, was made a valid species while *P putschkowskii* and *P vitellatus* were synonymised with others.

Recently Jaiswal (1957) while studying the trematode parasites of fishes and birds in Hyderabad State, added yet five more species *P dollfusi P leiopi*, *P moodliaheri P hyderabadensis* and *P singhi* and following Chauhan and Dollfus recognised in all thirty-one valid species excluding only *P ulcerolaedus*, *P longus* and *P longusmorbificans* but without assigning any reasons. On the other hand Yamaguti (1958) in his compilation has listed in all twenty-nine species along with three more as *P* sp Kotlan and Chandler (1927) *P* sp. Stafford (1931) and *P* sp Komiya 1951 As stated earlier most of these specific names had been suppressed by Witenberg *et* Eckman and Chen. Adding the species described by Jaiswal (1957) Yamaguti's number would swell to thirtyfour

On a perusal of the ten species described so far under this genus from Indian birds and a scrutiny of the differential features relied upon by the authors, it now appears that minor characters which in some instances exhibit a marked degree of overlapping, have mostly been used Thus *P indicus* Srivastava, 1938—a synonym of *P putschkowskii* according to Bhalerao *et* Gideon agrees essentially with the details of structure stressed by Witenberg *et* Eckman for the latter *P macroacetabulus* Chauhan 1940 also shows, in common with *P putschkowskii*, the characteristics regarding the uterine coils crossing the intestinal caeca laterally acetabulum being more than 1½ times the size of the oral sucker and vitellaria arranged in definite clusters. It is surprising that Chauhan preferred to distinguish his new species from others known to him on the characters emphasized alone in the key In *P macroacetabulus* the acetabulum is 1½ times larger than the oral sucker vitellaria are not restricted to post-acetabular region alone, cirrus sac reaches posteriorly the acetabulum, an extremely small oesophagus is present, heavy preacetabular coils of uterus are absent, ovary is dorsal to ventral sucker or greatly overlapping it and excretory bladder is "V" shaped— most of these characters, as already pointed by Witenberg *et* Eckman are extremely variable. Chauhan's contention regarding the extremely small oesophagus and the posterior extent of cirrus sac to the limits of acetabulum may have been due to the contraction of the specimen during fixation In extent, the vitelline clusters appear exactly like that given for *P putschkowskii*. The emphasis put on the absence of heavy preacetabular uterine coils cannot be of any diagnostic value as in a number of species including *P putschkowskii* the uterus infront of the acetabulum runs more or less a straight course. This condition has only been described for *P ovatus* The character regarding the dorsal position of the ovary and overlapping of the acetabulum is also present in a number of species including the specimens studied by Srivastava. The only character of some importance that remains is the "V" shaped excretory bladder. This, if really present, has a much greater systematic value and is possibly an error in observation.

Difficulty however is experienced in a true evaluation of the five new species dealt with by Jaiswal (1957). *P. dollfusi* Jaiswal, 1957 considered by the author to resemble *P. macrorchis* has been differentiated from it on the ratio between the suckers, in the position of the ovary in relation to acetabulum and testes and in the posterior extent of vitellaria—characters which it shares in common with *P. petuchkovskii* with which *P. macrorchis* had already been held identical by Witenberg et Eckman. There seems therefore no difference to warrant its retention as a separate species. Jaiswal stated that *P. lehupi* resembled *P. ovatus* in a number of characters. On account of absence of heavy preacetabular uterine coils, *P. lehupi* cannot be regarded to show resemblance to *P. ovatus* and in having the uterine coils laterally beyond the intestinal caeca with no coils infront of the acetabulum compact vitelleria without clusters extending posteriorly behind the middle of the body post-equatorial position of the testes, *P. lehupi* really resembles *P. cuneatus*. The character stressed in regard to the vitelline follicles overlapping partly the intestinal caeca can alone have no specific significance and may have resulted from contraction of the lateral margins of the body. *P. lehupi* is therefore suppressed as a synonym of *P. cuneatus*. The third species *P. hyderabadensis* has been stated to show closer affinities to *P. cuneatus* from which it has been separated as vitellaria posteriorly ending just behind the testes are smaller and the ratio between the two suckers is 1 : 3 which in *P. cuneatus* is 1 : 2. On a close scrutiny of these characters it appears that undue emphasis has been laid on such features which have been found to vary with the age of parasite and the state of contraction during preservation. Essentially *P. hyderabadensis* has compact vitellaria preacetabular uterine coils are entirely absent testes and posterior vitelline follicles lie in the posterior half of the body and acetabulum is more than 1½ times the size of the oral sucker—points which are also seen in *P. cuneatus* with which it is herein synonymised. The fourth species *P. mesolecithus* has been mentioned by Jaiswal as allied to *P. anatinus*. Witenberg et Eckman (1939) had listed *P. anatinus* as species inquirendae because of inadequate description and semi-diagramatic sketches which according to them indicated a juvenile specimen and expressing at the same time the view that it could be identical with *P. ...* Chen (1951) however has validated *P. anatinus*. As a detailed account of *P. anatinus* is not available it is not possible to comment on *P. mesolecithus* and *P. anatinus*. In *P. singhi* Jaiswal considered his species closer to *P. dollfusi* ... was differentiated on the sucker ratio being 1 : 1.5-2 the anterior ... of testes and the position of the ovary in relation to the ... testes ... acetabulum. This species was also separated from *P. ...* ... ded by Jaiswal mainly on the anterior extent of the vitellaria which ... in case of the former was stated to be slightly behind the intestinal ... while it was upto the intestinal fork in the latter and the ratio of the ... which was found to be 1 : 2.7 in *P. dollfusi* and 1 : ... in *P. ...* ... terior extent of vitellaria which is known to vary ...

some amount of stress from the author which cannot be justified Besides in the ratio of the two suckers Bhalerao et Gideon who had considered *P indicus* to be a synonym of *P putschkowskii*, gave it at 1 2 3 in their specimens, which is intermediate between that given for *P singhi* and *P indicus* Accordingly Jaiswal's *P singhi* is held as indentical to *P putschkowskii* *P folliculatus* which was believed to resemble *P singhi* had earlier been held a synonym of *P putschkowskii* by Witenberg et Eckman.

Thus, out of the ten species reported so far from India only two species *P putschkowskii* and *P cuneatus* appear to be valid ones.

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Summary

The trematode genus *Prosthogonimus* is briefly reviewed and of the ten species described from India only two viz *P putschkowskii* and *P cuneatus* are recognised. An illustrated brief account of the former is also given.

References

1 Alwar V S. & Lalitha C. M. (1961) A check list of the helminth parasites in the Department of Parasitology Madras Veterinary College Ind. Vet. J 38 (3) 142 148.

2. Bhalerao, G D. & Gideon, P W (1941) On the occurrence of *Prosthogonimus putschkowskii* Skrjabin 1913, in India *Rec. Indian Mus.*, 43 (4) 411-415.

3. Chauhan, B. S. (1940) Two new species of avian trematodes. *Proc Ind. Acad Sc.* 12 (3) 73-83

4. Chen, H. T (1951) *Prosthogonimus* from China, with remarks on the validity of the various species of the genus (Trematoda Plagiorchiidae) *Peking Nat. Hist. Bull.*, 19 (2 3) (1950) 183-192.

5. Dollfus, R. Ph. (1948) Sur les Prosthogoniminae trematodes de la bourse de Fabricius des oiseaux et leur bibliographie. *Mem. Mus. Nat. Hist. N S* 24 (1) 1 73

6. Hsu, Y C. (1935) Trematodes of fowls in Soochow *Peking Nat. Hist. Bull.* 10 (2), 141 150.

7 Jaiswal, G. P (1957) Studies on the Trematode parasites of fishes and birds found in Hyderabad State. *Zool Jahrb Syst.* 85 (1) 1 72.

8. Ku, C. T (1940) Studies on the genus *Prosthogonimus* of the domestic duck in Kwantung *Peking Nat. Hist Bull.* 15 (2) 119-131

9. Lal, M. B (1939) Studies in helminthology Trematode parasites of birds. *Proc Ind Acad. Sc.* 10 111-200

10 Lalitha C M. & Alwar V S. (1960) Parasites of domestic ducks (*Anas boschas domestica*) in Madras. (A preliminary note) *Ind. Vet. J* 37 (4) 179-181

11 Macy R. W (1934) Studies on the taxonomy morphology and biology of *Prosthogonimus macrorchis* Macy a common oviduct fluke of domestic fowls in North America *U. Minn. Technical Bull.* 89 71 pp

12. Nicoll, W (1924) The trematode parasites of North Queensland II. Parasites of birds *Parasitol.* 7 (2) 105-176.

13 Srivastava, H D. (1938) A new trematode *Prosthogonimus* n. sp. occurring in the oviduct of Indian fowls with remarks on Prosthogonimiasis *Ind. J. Vet. Sc. & Anim. Husb.* 8 (3) 123-220

14 Tubangui, M. A. & Masilungan, V A (1941) Trematode Parasites of Philippine vertebrates IX. Flukes from the domestic fowl and other birds. *Philipp J. Sci.* 75 (2) 131 142.

15 Witenberg G G & Eckman F (1939) On the classification of the trematode genus *Prosthogonimus* Vol. Jub. pro Prof. Yoshida 2, 129-143

16. Yamaguti, S. (1958) Systema Helminthum Vol. I Part I & II Interscience Publishers Ltd, London 1575 pp.

17 Yamaguti, S & Asada, J (1942) Trematodes of birds and mammals from Manchoukuo I. Bull. Inst. Scient. Res Manchoukuo. 6 (4) 490-512.

A CONTRIBUTION TO THE ALGAL FLORA OF GARHWAL

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Garhwal—5 629 sq miles in area is a hilly district of Uttar Pradesh. It lies almost entirely within the Himalayan mountain system between 29°26 and 31°5 north latitude and 78°12 and 80°6' east longitude with Tibet in the north, and the districts of Bijnor in the south Dehra Dun and Tehri in the west and Almora and Naini Tal in the east.

The district consist of successions of steep mountain ridges separated by deep glens. It is drained almost entirely by the Ganges river and its numerous tributaries. The climate varies very considerably from hot to extreme cold.

A record of the flora of the district, especially algae is scarce. During the past few years collections have been made from the central region of Garhwal which includes the Dudatoli ranges with a dense forest of several hundred square miles. There are a number of rivers such as Alaknanda Pindar Nayar and innumerable other rivulets and springs and the region therefore is rich in vegetation. The present paper deals with a part of the algal collection.

In all thirty seven forms have been described. Of these twentysix belong to Cyanophyceae and eleven to Chlorophyceae two varieties and one form are new

Systematic Enumeration of The Species Observed

CYANOPHYCEAE

I Chroococcales

Chroococcaceae

Genus *Chroococcus* Näg

(1) *Chroococcus schizodermaticus* West. Geitler in Rabenhorsts Kryptogamen flora von Europa, Band XIV Cyanophyceae, 1932, p 232 Fig 111b Forti in De Toni, Sylloge Algarum, 1907 5 15 Desikachary Cyanophyta 1959 p 103 pl 26, Fig 17

Lat. cell 7 8–12 48 (15 6) µ lat.cell. cum vag 14 82–21 84 µ; long cell cum vag 9 75—17 16 µ.

Habitat —Along with *Petalonema alatum* ttached to rocks etc. by the side of a rivulet Karanprayag—Adibadri road, March, 1957

forma minor forma nov

Lat. cell. 3 9–7 02 μ lat. cell cum vag 9 36 μ diam Colonies [illegible] 18 72 μ long and 9 36–13 26 μ broad.

The form occurs with the type specimen but varies from the type in having much smaller cells and colonies. The colonies moreover contain 2 cells only

Genus *Gloeocapsa* Kutzing

(2) *Gloeocapsa atrata* (Turp) Kutz Geitler op. cit. 1932 p 183 Forti, op cit., 1907 5 57 Desikachary op cit 1959 p 116 pl 24 Fig 8.

Lat cell 3 12–4 68 μ diam (rarely oblong up to 5 07 μ long) lat. cell cum vag 9 36–13 26 (–14 04) μ diam

Habitat.—Along with *Anabaena inaequalis* and *Calothrix gloeocola* in a spring Karanprayag—Adibadri road March 1957

(3) *Gloeocapsa nigrescens* Näg Desikachary op cit., 1959 p 117 pl. 24 Fig 15 17 Forti, op cit 1907 5 : 42

(a) Lat.cell 3 9–6 24 μ diam lat cell. cum vag 15 6–20 [illegible] μ diam

Habitat —Occurring along with *Petalonema alatum* attached to rocks by the side of a rivulet Karanprayag—Adibadri road, March 1957

(b) Cells 4 68–6 24 μ diam

The two forms differ from the type in having a broad yellowish brown sheath which is distinctly lamellated Form (b) further differs in having colonies with large number of cells or a number of 2 celled colonies embedded in a common gelatinous matrix each colony having a distinct lamellated sheath of its own The lamellations of the daughter colonies exhibit characteristic undulations within the parent colony

(4) *Gloeocapsa quaternata* (Breb) Kutz Geitler op cit 1932 p. 1[illegible] Fig 91e Forti op cit 1907 5 52 Desikachary op cit 1959 p 1[illegible]0 pl. [illegible] Fig 9

Lat cell [illegible] 2 73–) 3 12–4 29 μ diam lat [illegible]ll cum vag 6 [illegible]–8 [illegible] μ diam colonies up to 21 06 μ diam

Habitat – Along with *Scytonema myochrous* [illegible] attached to wet rocks on Lohba Panwaklal road April 1957

Genus *Gloeothece* Näg

(5) *Gloeothece sanguinea* Wille Geitler op cit 193[illegible] p 21[illegible] Desikachary op cit. 1959 p 108 pl 23 Fig 3

Lat cell 3 51–4 [illegible] μ long cell [illegible] μ [illegible] cell cum [illegible] (12 48–)15 6–21 84 μ diam

The form agrees with the type in the breadth and length of the cells but differs from the same in having a broad lamellated sheath which is usually coloured pale yellowish brown

Habitat.—Along with *Petalonema alatum* etc. attached to rocks by the side of a rivulet, Karanprayag—Adibadri road, March 1957

Genus *Aphanothece* Näg

(6) *Aphanothece microscopica* Näg Geitler op cit 1932 p 172 Fig 79 Forti op cit 1907 5 83 Desikachary op cit 1959 p 142 pl 22 Figs. 4 5 9

Lat. cell 4 29–5 07 μ long cell 5 46–7 8 μ.

Habitat.—Along with *Petalonema alatum* attached to rocks by the side of a rivulet, Karanprayag—Adibadri road, March 1957

II NOSTOCALES

Oscillatoriaceae

Genus *Oscillatoria* Vaucher

(7) *Oscillatoria laete-virens* (Crouan) Gomont Geitler op cit 1932 p 949 Fig 603e Forti op cit 1907 5 177 Gupta, The Algal flora of some paddy fields and its importance in soil economy Jour Res 1957 Vol 4 No 1 pl 1 Fig 8 Desikachary op cit 1959 p 213

Lat. trich 2 73–5 85 μ long cell 1 56–3 12 μ

Habitat.—On moist soil by the side of spring drain on way to Bendtal Karanprayag—Adibadri road, March 1957

(8) *Oscillatoria simplicissima* Gomont. Geitler op. cit. 1932 p 961 Forti op. cit. 1907 5 163 Desikachary op cit. 1959 p 221

Lat.trich 7 02–8 58 μ long cell. 3 12–4 68 (–6 24) μ

The form differs from the type in the presence of granules on either side of the septum.

Habitat.—By the side of an irrigation drain Karanprayag March 1957

(9) *Oscillatoria amoena* (Kutz) Gomont Geitler op cit 1932 p 969 Fig 603a, 611k Forti, op cit 1907 5 175 Tiffany and Britton, The Algae of Illinois 1295 p 344 pl 93 Fig 1073 Desikachary op cit 1959 p 230 pl. 40 Fig 12

Lat. trich 3 12–7 8 μ long cell. 1 56–4 68(–7 02) μ

Habitat – In a puddle by the side of Pindar river on moist soil by the side of flowing water Karanprayag—Adibadri road March 1957

(10) *Oscillatoria princeps* Vaucher ex Gomont Geitler op. cit 1932 p. 917

Figs 598a 601—cg Biswas Algal Flora of India and Burma 1919 p. 5? pl. 1 Fig 2 Desikachary op cit 1959 p 210 pl. 37 Figs 1 10 11 13 14 Singh K. P The Algal Flora of Vindhyan Formations of the Mirzapur District, *Proc. Ind. Acad. Sci. B* 49 1959

Lat. trich., 21 84–31 02 μ long cell. 2 34–4 29(–4 68) μ

Habitat —On moist soil Lohba April 1957

Genus *Porphyrosiphon* Kützing

(11) *Porphyrosiphon notarisii* (Menegh) Kütz exGomont Geitler op. cit 1932 p 986 Fig 631 Forti op cit 1907 5 314 Desikachary op. cit 1959 p 248 pl 47 Fig 9 Talpasayi The Myxophyceae of Kumaon Hills U P India Proc. Ind Acad Sci B 55 1962

Lat fil 12 48–15 6 μ lat trich. 9 36–10 92 μ long cell 6 21 10 9? μ

Habitat —On rocks Karanprayag March, *1957*

Genus *Microcoleus* Desmazieres

(12) *Microcoleus chthonoplastes* Thuret et Gomont Geitler op cit. 193? p. 1133 Fig 739 Forti op cit 1907,5 571 Desikachary op cit 19?? 343 pl 60 Figs 7 9

Lat trich 3 12–5 46 μ long cell. 3 12–9 36 μ

Habitat —On rocks etc forming a greenish black crust when dry Karanprayag—Adibadri road March 57

Nostocaceae

Genus *Cylindrospermum* Kütz

(13) *Cylindrospermum stagnale* (Kutz) Born et Flah Geitler op cit 193? p 819 Fig 520c Forti op cit 1907 5 472 Desikachary op cit 19?? p 363 pl 65 Fig 9

Var *Garhwalensis* var nov

Trichomes pale blue green constricted at the cross walls 3 1? 3 9 μ l oad cells barrel shaped 3 12–4 21 μ long heterocysts oblong ellipsoidal outer layer often gelatinizing broader than the trichome (4 6?) 5 07–7 8 μ broad and 5 85–9 36 μ long spores single subterminal at either end of the trichome long cylindrical yellowish brown 7 0? 11 31 μ broad and 17 ?? 31 3 μ long the epispore provided with distinct papillae

The variety resembles *C* ... and *C* ... in having spores with p... llose epispore but differs from them in having cylindrical spores. In the ... of the spore it resembles *C stagnale* but differs in the epispore ... ornamented The ornamentation of the spore wall in the variety in question further differs very markedly from *C stagnale* ...

venely striated and not papillose. The cylindrical spores with papillose epispore thus constitute the distinctive feature of the variety *Garhwalensis*

Habitat.—On moist soil Lohba, April 57

(14) *Cylindrospermum licheniforme* Kutz ex Born et Flah Geitler op cit. 1932, p. 821 Fig 520c Forti op cit. 1907 5 476 Tiffany and Britton op. cit., 1952 p. 362 pl 100 Fig 1146 Desikachary op cit. 1959 p 366 pl. 63 Fig 8.

Lat. trich., 2 75–3 1?(–3 9) μ long cell 3 12–6 24 μ lat. het. 3 12–4 68 μ long het., 3 12–5 46(–9 36) μ lat. spor., 9 36–14 04(–15 21) μ long. spor., 18 72–24 96(–30) μ. Spores rarely in series. Developing spores sometime show septation.

Habitat.—In shallow water in a puddle, Karanprayag—Adibadri road, March, '57

Genus *Nostoc* Vaucher

(15) *Nostoc ellipsosporum* (Desm.) Rabenh ex Born et Flah Geitler op. cit., 1932 p. 841 Fig 533 Forti op. cit. 1907 5 398 Desikachary op. cit. 1959 p 383 pl. 69 Fig 5 p 42 pl. 11 Figs 1-4

Lat. trich. 3 12–4 68 μ long cell. 4 68–10 9?(–17 16) μ lat. het. (4 29–)4 68–7 02 μ long het., (7 02–)8 58–15 6 μ lat. spor 3 46–8 58 (–14 82) μ long spor., 14 04–24 18(–29 64) μ.

Habitat.—In a water puddle along with *Cladophora glomerata* Karanprayag—Adibadri road, March 57

(16) *Nostoc sp* (sphericum ?) Vaucher ex Born. et Flah Geitler op cit. 1932 p 830 Fig 559b Desikachary op. cit. 1959 p. 390.

Lat. trich. 2 34–4 68 μ long cell. 2 34–3 9(–5 46) μ lat. het. 3 12–6 24 μ long het. 3 9–7 02 μ Spores not observed.

Habitat.—Attached to rocks in a spring, Karanprayag—Adibadri road, March 57

Genus *Anabaena* Bory

(17) *Anabaena laxa* (Rabehn) = *Anabaena inaequalis* (Kutz) Geitler op. cit. 1932, p. 896, Fig. 578 Fritsch The Genus Anabaena, with special reference to the species recorded from India and from th adjacent Asiatic mainland Jour Ind. Bot. Soc vol. XXVIII No. 3, 1949 p 155 Desikachary op. cit. 1959 p. 413

Lat. trich 3 51–4 68 μ long. cell 4 68–11 7(–14 04) μ lat. het. 4 68–6.24 μ long het. 4 68–10 14 μ lat. spor 5 46–8 19 μ long spor (6 24–)8 58–15 21 μ.

Habitat.—In a spring Karanprayag—Adibadri road, March, 57

(18) *Anabaena variabilis* Kutz. var *ellipsospora* Fritsch. Fritsch op cit., 1949 p 142–144 Fig 40–50

Lat. trich 3 51–4 68 μ long cell 4 68–8 58 μ lat. het., 5 07–6 63 (–8 97) μ long het 5 46–9 36(–11 7) μ lat. spor., 4 68–7 8 μ long spor 9 36–13 26(–15 6) μ.

Habitat.—On wet soil Karanprayag—Adibadri road, April 57

Genus *Aulosira* Kirchner

(19) *Aulosira implexa* Bornet et Flahault. Var *crassa* Dixit. Dixit The Myxophyceae of the Bombay Presidency India—I Proc Ind. Acad Sci. B, 3 1936, p 98 Fig 2A-D Desikachary op cit 1959 p 430 pl 80, Figs. 16-1…

Lat fil 22 62–28 08 μ Crass vag 1 56–3 9(–5 ?) μ lat. trich 14 04–17 16(–18 72) μ long cell., 2 34–7 8 μ lat. het 14 04–17 16 μ long het., 9 36–21 84 μ

Habitat.—Among other algae by the side of an irrigation drain Karanprayag March 57

SCYTONEMATACEAE

Genus *Scytonema* Ag

(20) *Scytonema chiastum* Geitler Geitler op cit 1932 p 750 Fig 473 Parukutty The Myxophyceae of the Travancore State, India Proc Ind Acad Sci. B 11 1940 p 119 Desikachary op. cit. 1959 p. 453 pl. 90 Fig 1

Var *minor* (including f *minor* Parukutty) var nov

Lat fil 18 72–28 08 μ crass vag., 3 12–6 24 μ lat. trich 12 48–17 94 μ long cell 1 56–7 8 μ lat. het., 14 04–15 6 μ long het. 7 8–10 92(–14 04) μ

The variety resembles *S chiastum* in the breadth of the filaments and in the sheath exhibiting parallel lamellation but differs in havin sparse pseudo branches and unconstricted trichomes It comes close to *S chiastum f minor* in the breadth of the filaments and the trichomes and length and bread of the heterocysts and cells but differs in (a) sparse pseudo branchin (b) un… stricted trichomes and (c) occurrence of undulated sheath at the apices of f… ments

Habitat —On stones in flowing water of a spring Karanprayag—A… badri road March 57

(21) *Scytonema ocellatum* Lyngbye ex Born et Flah Geitler op. cit … p 763 Fig 488 Forti op cit 1907 5 509 Desikachary op cit … p. 467 pl 92 Fig 3 Singh The Myxophyceae of the Kumaon Hills … India—I Proc Ind Acad Sci. B. 49 19 9

Lat. fil 10 92–20 28 μ lat trich. 7 8 14 04 μ long cell 3 9–1 … μ lat. het., 9 36–14 04 μ long het 7 8–10

Habitat.—On rocks forming a black crust when dry by the side of Karanprayag—Adibadri road, March 57

(22) *Scytonema myochrous* (Dillw.) Ag. ex Born et Flah. Geitler op. cit. 1932 p. 780 Figs. 49 501 502 Forti, op. cit. 1907 5 521 Desikachary op cit 1959, p. 487 pl. 90, Fig 3 and pl. 99 Fig 2.

Lat. fil., (10-92-) 15 6-32 76 (—40—56) μ crass vag (2 34-)4 68-7 8 (-12 48) μ lat. trich., 3 9-12 48 (-14 04) μ long cell., 3 12-7 8 (-18 72) μ lat. het. (6 24-)7 02-12 48 (-15 6) μ long het. (8 58-) 9 36-17 16 (23 08) μ.

Habitat.—On wet rocks, Lohba—Panwaklal road April, 57

Genus *Petalonema* Berk.

(23) *Petalonema alatum* Berk. Geitler op. cit., 1932 p. 789 Figs. 505 & 506 Desikachary op. cit. 1959 p 506, pl. 4 Figs. 1-3 12.

Lat. fil., 23 52-92 4 (-137 76) μ lat. trich. 4 68-10 92 μ long cell., 3 9-9 36 (-31 2) μ lat. het., 7 8-14 82 μ long. het. 11 7-22 62 (-30 81) μ

Habitat.—Attached to rocks etc. by the side of a rivulet Karanprayag—Adibadri road, March 57

RIVULARIACEAE

Genus *Calothrix* Ag

(24) *Calothrix gloeocola* Skuja. Desikachary op. cit 1959 p 542 pl. 109 Figs. 13-16, 18.

Lat. fil. 8 58-10 14 μ below 6 24-10 92 μ in the middle up to 5 46 μ towards the tip crass vag 1 56-2 34 (—5 07) μ lat. trich. 4 68-7 02 μ below 3 1 -3 9 μ in the middle, 2 34-2 73 μ at the tip lat. het. 4 68-7 02 μ long. het. 5 85-11 7 μ.

The form differs from the type in having a broader sheath which is usually yellowish or yellowish brown in colour

Habitat.—In the mucilage of *Anabaena inaequalis* in a spring Karan prayag—Adibadri road, March, 57

III STIGONEMATALES

NOSTOCHOPSIDACEAE

Genus *Nostochopsis* Wood em. Geitler

(25) *Nostochopsis lobatus* Wood. em Geitler Desikachary op. cit. 1959 p 570 pl 120 Figs 1-8 Forti op cit 1907 5 592

Lat trich. 2.34-4 68 μ long cell 4 68-18.72 μ lat. het (intercalary) 5 46-7 41 μ (intercalary) long het 6.24-9.36 μ lat het. (sessile) 5 46-6.24 μ long het. (sessile) 7 02—7 8 μ lat het (Pedicillate terminal) 3.9-6.24 μ len het. (Pedicillate terminal) 5 46-5 85 μ Sheath yellowish brown well defined, 12.5-39 μ broad

Habitat —By the side of a rivulet Lohba April 57

STIGONEMATACEAE

Genus *Stigonema Ag*

(26) *Stigonema mamillosum* (Ag) Hassall ex Born. et Flah Geitler op cit 1932, p 513 Figs. 315-317 Forti op. cit.,1907 5 502 Desikachary op. cit 1959 p 611 pl 137 Fig 1 Talpasayi, op. cit 1962

Lat. fil 24 96—49 12(—54 6) μ lat trich. 12 48-15 6 μ long cell, 3 63-11 7 μ diam lat het., 13.26 μ hormogones. 8.4-10.92 (—14.04) μ broad and 50 4 70.56(132 6) μ long

Habitat —Along with *Scytonema myochrous* on wet rocks Lohba—Panwakhal road, April 57

CHLOROPHYCEAE

CHLOROCOCCALES

Hydrodictyaceae

(27) *Hydrodictyon reticulatum* (L) Lagerheim Biswas, op. cit., 1949 p. 6 pl. 3 Fig 29 Gupta A contribution to the Algal flora of Khajiar-Chamba State Himachal Pradesh Proc. Nat. Acad Sci India Vol 20 Part III 1950 pp. 109-115

Cells 30 46-83 76 μ broad and 266.52-609 2 μ long

Habitat—In a water puddle formed by a spring Karanprayag—Adbadri road March 57

ULOTRICHALES

Ulotrichaceae

(28) *Ulothrix zonata* (Weber & Mohr) Kützing Biswas op cit 1949 p [illegible] Gupta op. cit. 1950

Cells 12 48-[illegible] 01 —53 76) μ broad and 13 44-3[illegible] [illegible] —[illegible] 4) μ lor

Habitat — Attached to stones etc In fast flowing water in Puna h [illegible] Rudraprayag on rock etc by the side of Ramganga river Adbadri March

CLADOPHORALES

Cladophoraceae

(29) *Cladophora glomerata* L.) Kutzing Biswas op cit 1949 p [illegible] pl. [illegible] Fig 49a b Gupta op cit 1950

Cell 30 46-137 07 μ broad and [illegible] (—[illegible]) μ long branches bushy at the top filaments bright green.

Habitat.—In a rivulet Rudraprayag attached to stones by the side of Pindar river Karanprayag March, 57

CHAETOPHORALES

Chaetophoraceae

(30) *Stigeoclonium aestivale* (Hazen) Pascher's Susswasser flora.

Cells of the main filament 5.85-10.14 μ broad and 7.8-28.08 (-31.2) μ long cells of branches 3.12-6.24 μ broad and 5.46-26.52 μ long

Habitat.—Attached to stones in flowing water Karanprayag March 57

OEDOGONIALES

Oedogoniaceae

Two species of *Oedogonium* have been collected but the specific identification was not possible as they were in vegetative condition only

(31) *Oedogonium sp*

Filaments 30.24-33.6 μ broad cells 47.04-67.2 μ long

Habitat.—In standing water of a stream along with *Spirogyra polymorpha* Karanprayag March, 57

(32) *Oedogonium sp*

Filaments 10.08-11.76 μ broad cells 36.96-60.48 μ long

Habitat.—In standing water of a stream along with *Cladophora glomerata*, Karanprayag, March 57

CONJUGALES

Zygnemaceae

(33) *Spirogyra fuergensii* Kutz Tiffany and Britton, op cit 1952 p 146 pl 41 Fig 463 Randhawa, Zygnemaceae, 1959 p. 294 Fig 230.

Lat. veg cell. 26.88-30.24 (36.96) μ long veg cell. (33 6-)40.3? 124.32 (201 6) μ lat spore 31.2 32 76 (—37 44) μ long. spore (38.22) 51 48-79.56 μ

Habitat —In a puddle by the side of Pindar river Karanprayag March, 57

(34) *Spirogyra singularis* Nordstedt. Tiffany and Britton op cit 1952 p 146 pl. 43 Fig 454 Randhawa op cit., 1959 p. 295, Fig 231

Lat. veg cell (16.8-) 30.24-36.96 μ long veg. cell. 53 76-178.08 (—241.92) μ lat. spore 28.08-37 44 (-41 12) μ long spore 41.34-82.68 μ.

Habitat.—In a puddle by the side of Pindar river Karanprayag March '57 in a puddle by the side of Alakananda river in shallow water puddles in river beds Adibadri, March 57

(35) *Spirogyra lutetiana* Petit Randhawa op cit 1959 p. 308 Fig 273.

Lat. veg cell 30.24-47 04 (33 76) μ long veg cell 57 1*-131 4 (215 04) μ lat spore. 41 12 53 04 μ long spore., 49.92 71 76 μ

Habitat.—In standing water of a stream Karanprayag March 57

(36) *Spirogyra polymorpha* Kirchner Randhawa op. cit 1959 p 309 Fig 276.

Lat. veg cell 26 88-30.24 (-33 6) μ long veg cell. 47 04-190.24 μ lat spore 29 64-35 88 μ long spore 49 92-60 06 μ.

Habitat —In standing water of a stream Karanprayag in a drain by the side of Karanprayag—Adibadri road March 57

(37) *Sirogonium sticticum* (Engl. Bot) Kutzing Tiffany and Britton, op. cit., 1952 p 162 pl. 50 Fig 531 Randhawa op cit., 1959 p 495 Fig 500.

Lat. veg cell 45.36-53.76 μ long veg cell 70.56-171.36 μ lat spore., 63 96 μ long spore 104.52 μ

Habitat —In a stream Lohba April, 57

Acknowledgments

Our thanks are due to the Agra University for providing financial assistance and to Dr K. P Bhatnagar Ex Vice-Chancellor of the University for the interest taken in the investigation

Plate I

1 Chroococcus schizodermaticus West
2 Chroococcus schizodermaticus West forma minor forma nov
3 Gloeocapsa nigrescens Näg a, b.
4 Gloeothece samoensis Wille.
5. Oscillatoria laete-virens (Crouan) Gomont a b
6. O simplicissima Gomont a b

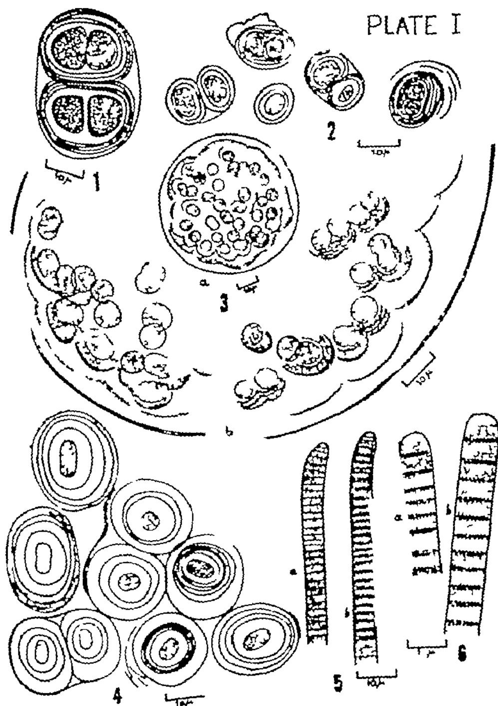

PLATE I
1
2
3
a
b
4
5
a
b
6
a
b

PLATE II

7 Cylindrospermum stagnale (Kütz) Born et Flah var Garhwa nov
8. Scytonema chiastum Geitler var minor var nov a b
9 Petalonema alatum Berk.
10 Nostochopsis lobatus Wood em Geitler

EFFECT OF SOIL MOISTURE ON ROOT ROT OF GUAR (*CYAMOPSIS PSORALIOIDES* DC.) AND WILT OF GRAM (*CICER ARIETINUM* L.) CAUSED BY *SCLEROTIUM ROLFSII* SACC.*

S B MATHUR

Botany Department Agra College Agra

INTRODUCTION

Garrett (1944) has given a consolidated list of diseases favoured by low or high moisture content of the soil. Most wilt diseases caused by species of *Fusarium* are favoured by high soil moisture (Goss 1921 and 1923 Clayton 1923, Tharp and Young 1939 Strong 1946, Subramanian 1950 and Chauhan 1959) Among the diseases favoured by low soil moistures are seedling blight of wheat and corn (Dickson *et al* 1923) potato scab (Sanford 1923) tomato wilt (Foster and Walker 1947) and *Fusarium* disease of broad beans (Yu and Fang 1948)

In this paper results are presented on the effect of soil moisture on root-rot of guar (*Cyamopsis psoralioides* DC.) and wilt of gram (*Cicer arietinum* L.) both caused by *Sclerotium rolfsii* Sacc.

METHOD AND MATERIAL

The experiments were conducted in pot cultures. Four moisture levels viz., 10, 15 20 and 25% were maintained on oven dry basis of soil and ranged well within the water holding capacity of the soil, which was determined following Keen-Raczkowski's method as described by Piper (1944)

Six pounds of garden soil was filled in pre-weighed glazed metal pots (8¾ X 7"). The inoculum of the pathogen was raised on autoclaved corn-meal sand medium and equal quantities of the same were mixed thoroughly with the soil in experimental pots seven days before sowing Five replications (5 pots) were maintained for the infested series and only one pot for the control in each treatment. Six seeds were sown per pot after seven days of infestation The experiments were performed in a glass house. The moisture levels were kept constant by weighing each pot every day and adding water to maintain the original weight. Observations on percentage of seedling emergence and of post-emergence seedling mortality were recorded 4 days after sowing for 44 days at regular intervals of 4 days The data obtained were analysed statistically following analysis of variance method. The pathogen was reisolated from the infected collar region of the plants to make sure the cause of their death due to the parasite The seedlings in the control pots remained healthy throughout the period of investigation in each treatment.

* A part of Thesis approved for the Ph. D. degree of the Agra University Agra.

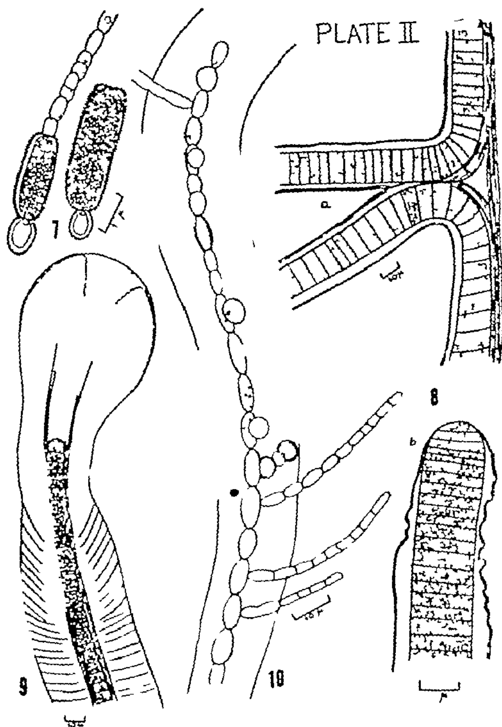
PLATE II

7 Cylindrospermum stagnale (Kütz.) Born et Flah var. Gathwalensis var. nov.

8. Scytonema chlastum Geitler var. minor var. nov. a. b.

9 Petalonema alatum Berk.

10 Nostochopsis lobatus Wood em. Geitler


PLATE II
7
8
9
10
a
b

EXPERIMENTAL STUDIES ON THE OVIPOSITION BEHAVIOUR OF *OMOCESTUS VIRIDULUS* (LINN.) (ORD. ORTHOPTERA)

J. C. Basu Choudhuri*

Introduction

Richards and Waloff (1954) have made extensive field observations on the distribution of egg-pods of *Omocestus viridulus* (Linnaeus) along with *Stenobothrus lineatus* (Panzer) at Silwood Park, Sunninghill, Berkshire (England). The authors have also observed that *O. viridulus* usually deposit their egg pods at the base of grass-blades or in association with other plants such as *Calluna sp.* *Erica sp.* and *Galium sp.* Some egg-pods were also found in thick patches of moss. These two species lay their egg-pods exclusively in soil covered with vegetation although some of their pods were found in bare ground with very sparse vegetation. The oviposition response of *O. viridulus* were studied in laboratory to find out the factors which determine the choice of oviposition sites.

Methods and Materials

Experimental insects. The hoppers were collected from the field and were reared under laboratory conditions; they were fed on grass (*Holcus* sp. and *Agrostis* sp.). Insects were allowed to mate under cage conditions before using them for experiments. But later in the season gravid females were collected from the field for laboratory experiments.

Cages. The cages (21×16×19 in.) were made of wood with glass sides, hard board bottom and trap door in front. About 1.5 in. from bottom a false-bottom was provided on which treatments in petri-dishes or glass tubes for egg-laying were placed; this false bottom could be slided in or out without disturbing the experimental insects.

The hoppers were reared in standard entomological (Watkins and Doncaster) celluloid cages.

Procedure and Design of experiments. The experiments were carried out in cages which were kept in the constant-temperature room (T 70 C R.H. 55%). Experimental materials were placed either on petridishes (3.5 ×0.5 in.) or in small glass vials (2×1 in.). Experiments were carried out with fully mature gravid females. For every experiment fresh batch of gravid females were used. The treatments for observing oviposition response were placed in the cage in replicates at random using the Random Number tables. The usual period of exposure was 8 hours. After this period the treatments were taken out and examined for the egg pods. During the experiments electric

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lamp (60 W) of the cage was always switched on to make uniform lighting within the cage In each experiment ten gravid females were used otherwise the number has been stated

At the beginning of the season some preliminary experiments were carried out and on their basis improved experiments were planned (the results of the latter are only included here) The experiments designed were of single factor type on account of scarcity of gravids females in the late season it was not possible to design multiple factorial experiments to study the influence of the interactions of various factors studied individually

Experiments and Observations

Field observations on *O* *cindulus* suggest that these insects exhibit a association with short vegetation especially grasses for egg-laying The following vegetational factors which may influence the choice of oviposition sites were considered

1 Stand factor of the vegetation
2 Influence of food plants
3 Humidity of grass/food plants
4 Surface-texture of vegetation
5 Colour of vegetation
6 Odour of vegetation

Influence of the "Stand" Factor

Common grasses (*Holcus* sp., *Festuca* sp and *Agrostis* [illegible]) were collected from the natural habitat placed in petri-dishes and offered for egg-laying to gravid females of *O* *cindulus* The treatments were

1 Green grass with long blades planted in wet sand
2 Dried grass with shrivelled blades planted in wet sand
3 Long green grass clipped close to the upper end of sheath [illegible] late [illegible] appearance (as observed in the field after grazing by cattle
4 T[illegible] mown lawn grass cut into a circular mat with a sharp [illegible]
5 Dead and dried grasses which were piled on petri-dishes [illegible] ten[illegible]d
6 Wet and well mixed with dead plant [illegible] l[illegible] and torn

[illegible] treatments with their replicates were offered to twenty [illegible] females the activities of the insects were frequently observed [illegible]

The number of eggs laid in different treatments [illegible] (Table [illegible]) The percentage of [illegible] was about 3 [illegible]

long blades, stubbly-grass, dried-moistened grass and turf respectively. No egg-pods were found on the long dried grass and in the wet-sand treatments. The numbers stated in the table indicate a preference; the data were subjected to the X^2 analysis. The result of analysis (P=0.001) points out that the egg laying was definitely non-random; it also suggests that there exists some relationships between the egg-laying and the choice of oviposition sites.

Further the data also indicate that the gravid females of *O. viridulus* preferred to lay egg-pods on the blades of grass (about 65%) including green and moistened dried grasses. It shows therefore the humidity of grass or grass itself has some influence on the choice of oviposition sites. On several occasions, the females were seen visiting the dried grass which was kept close to the fresh green grass but no egg pods were laid on the former. In another experiment, it has been observed that whenever the long dried grass treatment was thoroughly moistened few egg pods were found on it. Thus a strong preference for humidity of grass is shown. It is also possible, that the rigidity, surface-texture and palatableness of the grass may also contribute important factors in the selection. In every case, the egg-pods were found well above the surface level of sand; the usual height at which oviposition took place was 1.0-1.6 in.

Influence of Food-Plants

The odour of food plants in many insects as corn cutworm, *Chlorides obsoleta* Fabr., (McColloch, 1922) and citrus borer *Citripestis sagittiferella* Moore (Pagden, 1931) induces gravid females to lay eggs on the vegetative parts of the plants. With these ideas in view an experiment was planned to observe the oviposition response of *O. viridulus* to different plants occurring in their natural habitats. The following plants were collected and offered for egg-laying :—

1. *Holcus lanatus* L.
2. *H. mollis* L.
3. *Agrostis tenuis* Sibth.
4. *Festuca rubra* L.
5. *Dactylis glomerata* L.
6. *Lolium perenne* L.
7. *Arrhenatherum elatius* Mert. & Kock.
8. *Erica cinerea* L.
9. *Rumex acetosella* L.
10. *Achillea millifolium* L.
11. *Plantago lanceolata* L.
12. *Lathyrus pratensis* L.
13. *Juncus* sp.

These plants were kept fresh by placing them in glass vials containing water. A control (strips of wood-shavings were twisted together and tied to

make a tuft of grass) was also used. Notes were kept of the sites, manner of attachment to the leaves and the height at which the egg-pods were laid.

The number of egg-pods laid on different plants is summarized in Table 2. The percentage of oviposition on *Holcus*, *Festuca* and *Dactylis* were 22, 18 and 16 respectively. The percentage of egg laying on other plants was very low. No pod was laid on the control. The data clearly indicate that these insects have laid more egg-pods on grasses (*Holcus*, *Festuca* and *Agrostis*) and either have laid very few egg pods or none on other plants. *Holcus* and *Festuca* are dominant species of grass and are softer and more succulent than other species of grasses occurring in the habitats of *O. viridulus*. The egg-pods were invariably deposited near basal ends of the blades close to the mouths of vials in which the grasses were kept standing in water. These observations suggest that to a certain degree some preference have played in depositing egg-pods near the vicinity of relatively high humid "pockets" *i.e.*, on the grass blades and near the mouth of vials where evaporation rate is expected to be higher hence more humid than other "pockets" in the cage. The X^2 test was applied to the data in Table 2. The calculated value of X^2 is 57.14 which for 13 degrees of freedom is greater than that for 0.1 probability level. The analysis suggests that the egg-laying of *O. viridulus* does not occur at random on the plants presented to them.

Further experiments were made to study the influence of humidity, surface-texture, colour and odour of grass in selecting oviposition sites.

Influence of Humidity

To study the effect of humidity of grass on the oviposition response of *O. viridulus* the following experiments were designed.

Experiment I. Tufts of *H. lanatus* and *F. [illegible]* were thoroughly dried in an oven till the blades became shrivelled. The dried grasses were divided into two batches—one was well soaked in water and the other was kept dry. Both the batches were made of equal proportions of dried *H. lanatus* and *[illegible]*.

The data are represented in Fig. 1. About 22 [illegible] and [illegible] of egg-pods were laid on dry and moistened grass respectively. The *t* test was applied to the data (Table 3). The calculated value of *t* is ?.71 which [illegible] at the [illegible] probability level. The analysis suggests an [illegible] between the preference for oviposition and humidity of grass.

From this experiment it may be inferred that an [illegible] female [illegible] for damp and moist vegetation to lay its egg [illegible] genuine selection of [illegible]. To check [illegible] by the [illegible] and the species of grass in the selection of oviposition [illegible] experiment was designed.

Experiment 2. The experiment aimed at to find out whether (a) the humidity of grass (b) the effect of grass-species or (c) the combined effect of both have any influence on the choice of oviposition sites.

It has been observed that in the food-plants experiment a large number of egg-pods were laid on *H. lanatus*, *F. rubra* and *D. glomerata*. These grasses were selected as experimental materials. The tufts of *Holcus*, *Festuca* and *Dactylis* were collected from the natural habitat and were divided into two batches—(i) one was well dried in an oven and (ii) the other was kept in its natural condition green and wet. The treatments with replicates and a control (strips of wood shavings twisted into tufts) were offered in the cage.

The number of egg pods laid in different treatments is represented in Fig. 2; a distinct preference for oviposition on fresh and moist grasses especially *Festuca* is shown. The data (Table 4) were subjected to the analysis of variance test. The full analysis is summarized in Table 5. The following conclusions are derived:

1. One of the main factor—the humidity of grass is significant. About 89% of egg-pods were laid in the wet treatments and only 11% in the dry treatments.
2. The second main factor—the species of grasses is not significant.
3. The interaction between the main factors, the humidity—grass ($m \times p$) species is also non-significant.

Influence of Surface-Texture

The experiment was designed to observe the effect of contact stimulus on the oviposition response and in the selection of egg-laying sites. The following materials were used to provide different textures for egg laying surfaces.

1. Wood shavings (about 6 in. long)—stripes of wood shavings were twisted together, tied and made into a thick tuft simulating grass. The surface texture was similar to the grass with coarse blades.

2. Pipe-cleaners (6.5 × 0.5 in.)—a bunch of ten cotton pipe-cleaners were tied together and were inserted through cork fitted into a glass-vial. The surface texture was soft and silky.

3. Bundle of strings (6 in. long)—about fifty pieces of string were grouped around a core of thin glass rod (6 × 0.1 in.) free ends were tightly fastened. The surface texture was lightly rough resembling the tufts of *Festuca*.

4. Glass rod (6 × 0.1 in.)—a glass rod was inserted in a cork fitted to a vial. The surface of the glass rod was very smooth; five narrow rubber rings of 1 cm. in length were slipped over the rod. These rings were placed at equidistance on exposed length of the rod. These

rings provided steps for climbing females and also rough spots on smooth surface.

These "artificial grasses (Fig 3) were soaked in water and kept standing in glass-vials. No food was kept in the cage during the experimental period to avoid the influence of food-plant.

The number of egg pods laid in different treatments is tabulated (Table 6). The percentage of oviposition on the wood shavings, bundle of strings, pipe-cleaners and the glass-rod was 59, 22, 12 and 7 respectively. The bulk of egg pods was laid on wood-shavings whose texture and appearance approach close to natural grasses. It seems that these insects showed some preference for coarse surface texture as is evident from low number of egg-pods on silky (pipe-cleaners) and smooth (glass-rods) surfaces. On the glass rods, pods were found attached to rubber rings and no pod was found attached to the glass surface. Only 22% of egg pods were laid on the bundle strings as compared to 59% on wood shavings; this suggests the treatment (No 3) was not much favoured. The possible explanation for this may be due to the fact that when the bundles of strings are moistened they become soft and shoggy. Since these could retain moisture for longer period the humidity around the strings was high as compared to other treatments which might have attracted gravid females to deposit their egg pods on them even though the surface-texture had become soft. Moreover soft and shoggy conditions of the string bundles proved to be ideal substratum for releasing "probing" response.

The data were subjected to statistical test, the calculated value of X^2 was 26.4 which is significant at the 1% level. The analysis suggests that egg-laying in the experiment proves to be non-random. Humidity along with the surface texture of artificial grasses may have determined and influenced the selection of suitable egg laying sites.

Influence of Colour of Grass

The colour of grass (food-plants) may also provide a stimulus to gravid females in the selection of oviposition sites under natural conditions. Tufts of wood shavings were dyed green and black by dipping them in aqueous solution of appropriate dye. The treatments with their replicates were moistened thoroughly before offering them in the cage.

The data are given in Table 7. The number of egg-pods dep[illegible] on green, black and control treatments was 10, 12 and 13 respectively. The differences are evidently not significant; it may be inferred that the [illegible] does not influence the oviposition response to a great extent.

Influence of Odour of Grass Extract

This experiment was performed to study [illegible] effect of the [illegible] grass extracts on the choice of oviposition sites. The [illegible]

were impregnated by dipping them in a concentrated aqueous solution of the grass-extract prepared by crushing a large amount of *H. lanatus*. The control was soaked in plain water.

The number of egg-pods laid on the treated and control tufts was not very significant—11 and 19 pods respectively. Perhaps the odour of grass does not release any stimulus to the gravid females. Since, no study was carried out on the olfactory sense of gravid females of *O. viridulus* with olfactometer hence, the point remains doubtful and needs further investigation.

Discussion

Waloff (1950) has classified *O. viridulus* as purely hygrophilous species. Also, field observations (Waloff, 1950; Richards and Waloff, 1954) point out that *O. viridulus* usually lay their egg-pods well above the soil on grass blades (*Holcus*, *Agrostis*, and *Festuca*) thick patches of moss or other plants (e.g., *Calluna* sp., *Erica* sp. and *Galium* sp.) found in their habitats. At times the egg-pods of *O. viridulus* are also found in bare ground with sparse vegetation. These observations suggest that *O. viridulus*, like other grasshoppers may exhibit a preference for oviposition sites as studied by Choudhuri (1958) in case of *Chorthippus parallelus* (Zetterstedt) and *Chorthippus brunneus* (Charpentier). In relatively few species of grasshoppers the oviposition is usually associated with plant tissues as observed in *Hieroglyphus banian* Fabr., (Coleman and Kanan 1911), *Oxya* sp. (Rao 1921), *Chrysochraon dispar* Germ. (Shapovalov, 1923a; Ramme, 1927), *Chloealtis conspersa*, Harr., *Napodimopsis abdominalis* (Thom.) (Criddle 1933), *Chorthippus albomarginatus* (Degeer) and *Mecostethus grossus* (Linnaeus) (Waloff, 1950).

The laboratory observations indicate that *O. viridulus*, always laid their egg-pods on various treatments (different plant species and other plant simulating materials) well above the surface, a necessary requirement pertaining to "stand" factor. The trailing lawn-grass and scattered plant debris will not attract females to deposit their egg-pods in them if a good standing crop of *Holcus* or *Festuca* is available within their habitats. Further the foodplants experiment suggests that the gravid females are attracted towards food-plants which is shown by their preference for depositing a fairly good percentage of egg-pods on *Holcus lanatus*, *Festuca rubra*, *Agrostis tenuis* and *Dactylis glomerata* in presence of about dozen of plants which are commonly found in their habitats. During their search for food plants when they come across a suitable site depending on humidity and surface texture of the grass the oviposition response is released and they oviposit their egg-pods. In the selection of the oviposition sites humidity of grass plays important and significant role, the texture of grass is also considered. The substratum which facilitates probing will be obviously preferred as seen in cage experiments, the wood-shavings simulating grass and the bundle of strings were selected to lay egg-pods as compared to the glass-rods and the pipe-cleaners. These requirements explain the ecological needs of grass-blades, and the occurrence of egg-pods of *O. viridulus* in the vic[illegible]

TABLE 1

The number of egg-pods laid by O. viridulus on grass

Sl. No.	Treatments (grasses)	Number of egg-pods	Percentage of egg-pods
1	Green grass with blades	15	33
2	Dried long grass	0	0
3	Clipped grass	12	30
4	Moistened dried grass	11	27
5	Turf	2	5
6	Wet sand mixed with dead plant tissues	0	0
	Total	40	100

TABLE 2

The number of egg-pods laid by O. viridulus on different plants

Sl. No.	Name of plants	Number of egg-pods	Percentage of egg-pods
1	*H. lanatus*	13	22
2	*A. tenuis*	8	13
3	*F. rubra*	11	18
4	*D. glomerata*	10	16
5	*L. perenne*	[illegible]	3
6	*A. elatius*	0	0
7	*F. ovina*	4	7
8	*C. vulgaris*	4	7
9	*P. [illegible]*	0	
10	*P. lanceolata*	7	
11	*L. [illegible]*	1	
12	*Juncus sp.*	0	
13	Wo[illegible]	0	
	Total	[illegible]	

TABLE 3

The number of egg-pods laid by O. [illegible] on grasses kept under different moisture conditions

No. of experiment	Dried grass	Moistened fresh grass	Total egg-pods
1	1	[illegible]	[illegible]
2	0	[illegible]	8
3	4	[illegible]	[illegible]
4	0	1	1
5	3	8	11
6	1	6	[illegible]
7	0	4	[illegible]
Total	9(22%)	31(78%)	[illegible]

TABLE 4

The number of egg-pods laid by O. [illegible] on grasses [illegible] moisture conditions

Species of grass	Dry grass	Green grass
Holcus sp.	1	
Dactylis sp.	4	13
Festuca sp.	0	[illegible]
Wood shavings	0	[illegible]
Total	5	

TABLE 5

Analysis of variance applied to square root transformations of the data given in Table 4

Source of variance	Degrees of freedom	Sum of squares	Means of squares	Variance ratio
Moisture (m)	1	57 04	57 04	12. 1
Plants (p)	3	33 79	11.26	2 4 n.s.
m x p	3	39 49	13 16	2 8 n.s.
Residue	16	74 64	4 66	
Total	23	201 96	86 12	

Denotes significant t the 1 probability level.
n. s. denotes not significant.

TABLE 6

The number of egg-pods laid by O viridulus on different kinds of materials

Sl. No.	Materials	Number of egg-pods	Percentage of egg-pods
1	Pipe-cleaners	5	12
2	Glass-rod	3	7
3	Wood shavings	4	59
4	Bundle of strings	9	22
	Total	41	100

TABLE 7

The number of egg-pods laid by O viridulus on different coloured wood shavings

Sl. No.	Wood-shavings	Number of egg-pods	Percentage of egg-pods
1	Black	10	[illegible]
2	Green	12	35
3	Unc loured	13	[illegible]
	Total	35	[illegible]

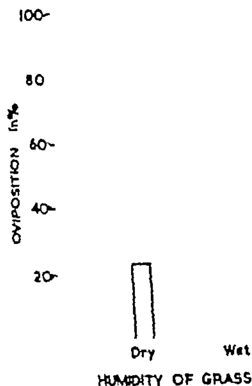


FIG 1 THE PERCENTAGE OF EGG-PODS LAID BY O VIRIDULUS ON GRASS OF DIFFERENT HUMIDITIES

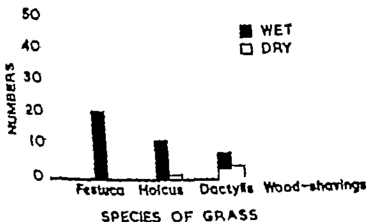


FIG 2 NUMBER OF EGG-PODS LAID BY O VIRIDULUS IN DIFFERENT SPECIES OF GRASS KEPT UNDER DRY AND WET CONDITIONS

TABLE 5

Analysis of variance applied to square root transformations of the data given in Table 4

Source of variance	Degrees of freedom	Sum of squares	Means of squares	Variance ratio
Moisture (m)	1	57 04	57 04	12 24
Plants (p)	3	33 79	11.26	2.4 n.s.
m x p	3	39 49	13 16	2 8 n.s.
Residue	16	74 64	4 66	
Total	23	204 96	86 12	

Denotes significant at the 1% probability level.
n. s. denotes not significant.

TABLE 6

The number of egg-pods laid by O viridulus on different kinds of materials.

Sl. No.	Materials	Number of egg-pods	Percentage of egg-pods
1	Pipe-cleaners	5	12
2	Glass-rods	3	7
3	Wood shavings	24	59
4	Bundle of strings	9	22
	Total	41	100%

TABLE 7

The number of egg-pods laid by O viridulus on different coloured wood shavings

Sl. No.	Wood-shavings	Number of egg-pods	Percentage of egg-pods
1	Black	10	28
2	Green	12	35
3	Uncoloured	13	37
	Total	35	100%

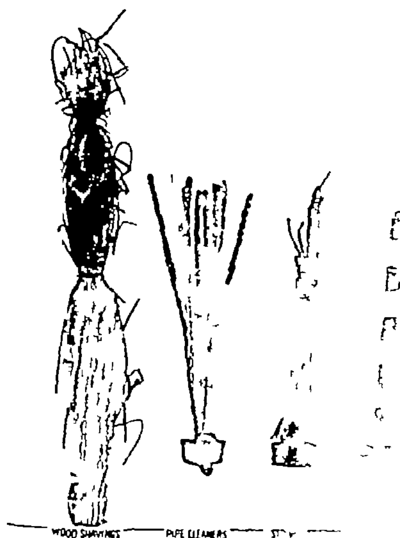


Fig. 3. Different kinds of materials used to stow ...

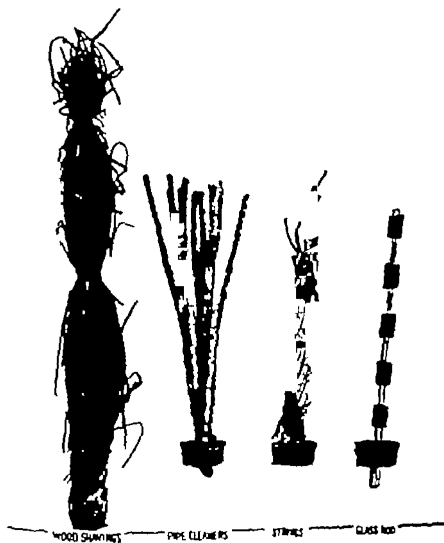


Fig. 3 Different kinds of materials used to simulate natural grass.

OBSERVATIONS ON THE OVULATION AND CORPUS LUTEUM FORMATION IN *BAGRADA CRUCIFERARUM* KIRK. PENTATOMIDAE HETEROPTERA*

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Contents

INTRODUCTION

From the literature only Wigglesworth 1936, Bonhag and Wick 1953 Singh 1954 Ray and Dasgupta 1956 have described corpora lutea in various Heteroptera, although this structure has been observed by many workers in the course of their morphological studies in several insect orders none of them except Singh 1954 in *Dysdercus fasciatus*, Singh 1958 in *Locusta migratoria* and *Schistocerca gregaria* and Singh and Nayar 1961 in *Coccinella septempunctata* have given time relation between ovulation and corpus luteum formation. It is not clear from the literature whether ovulation and oviposition behaviour is in some way connected with the type of ovarioles. In order to investigate some of these facts, the authors have studied ovulation and corpus luteum formation in some detail in *Bagrada cruciferarum*.

TECHNIQUE

The last instar nymphs of *Bagrada cruciferarum* were collected from the fields around Agra. These were kept in glass jars (10" × 8") covered over by wire gauze, which were put in a constant temperature room at 28° C and 55-60% R.H. Newly emerged adults were separated into pairs and a male and female of known ages were kept in a small glass tube (3 × 1") which were covered with muslin Insects were fed on fresh raddish leaves twice daily The tubes were examined for oviposition at 10.00 a.m. in the morning and at successive intervals of two hours upto 8.00 p.m. Eggs were mostly laid at night

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hours on muslin rarely on leaves or on the walls of the tube. A record of the history of these pairs was kept.

Female bugs were narcotized in Ether and were dissected in Ringer's solution right from the 1st day of emergence to the day of maturation (7th day) to study the progressive development of the ovarioles. Dissection was continued just after and within twenty four hours of the 1st oviposition then at subsequent interval of one day until the 2nd batch of eggs was laid, and also within twenty four hours of successive ovipositions. Immediately after the dissection the ovarioles were transferred to freshly prepared Bouin's fluid 8 μ thick sections were cut Ehrlich's Haematoxylin Eosin and Mallory's triple stains were used for staining

Description of the Adult Ovary

The ovaries are whitish and each composed of six acrotrophic ovarioles, which open into the lateral oviduct of their side through a pedicel. On each side the ovarioles anteriorly end in terminal filament, which unite to form a median ligament. In a newly emerged insect the ovarioles are broader at the tip and measure 0 93 mm in length (excluding terminal filament) Vitellarium and germarium are not distinguishable. Each ovariole progressively increases in size and on the 7th day measures on an average 1 8 mm (Graph 1) Germarium and vitellarium can be distinguished from the 4th day onwards, where the colourless basal egg measures 0 28 mm, the basal plug measuring 0 12 mm is also delimited The egg_1 progressively increases in size till on the 7th day measures 0 82 mm (Graph 1) At this stage egg_2 and egg_3 were also distinguished (Fig 1) In some ovarioles egg_1 and egg_2 were observed maturing at the same rate.

Oviposition

Copulation commences soon after the emergence of the adult and the process goes on continuously if the male is not separated from the female and interrupted only during oviposition.

In certain cases, when the insects were dissected seven days after the emergence, the eggs were found either in the pedicel or in the lateral oviduct, hence presumably oviposition takes place some time after the ovulation.

The oviposition in 92 insects (Table 1) reveal that the minimum time for maturation is between 7 18 days. The insects ovipositing before and after these days were regarded as abnormal.

TABLE 1

Days after emergence	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Number of females laying 1st batch of eggs	1	2	2	6	14	4	12	14	7	6	5	4	4	5	3	2	1

TABLE 2

Showing the oviposition in successive batches

Batches / Insects	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
A	12(8)														
B	5(7)														
C	12(9)														
D	7(8)														
E	10(10)														
F	8(16)														
G	9(11)														
H	4(14)														
I	4(8)														
J	11(8)														
K	6(8)														
L	9(9)	5(3)													
M	8(8)	7(1)													
N	(10)	12(1)	12(1)												
O	19(9)	10(2)	7(1)												
P	4(8)	10(3)	10(2)	13(1)											
Q	3(8)	7(2)	5(1)	11(1)											
R	12(10)	8(1)	12(1)	13( )	10(2)										
S	9(13)	9(1)	5(2)	2(1)	6(1)	6(1)									
T	11(11)	11(2)	4(2)	10(1)	10(2)	11(2)	11(1)								
U	4(8)	7(1)	24(1)	12(2)	12(1)	12(2)	11(1)	14(1)							
V	12(9)	14(1)	9(2)	9(1)	11(1)	12(1)	10(5)	7(4)	11(2)						
W	7(12)	3(5)	6(1)	14(1)	7(1)	5(1)	6(1)	6(1)	16(1)	2(1)					
X	10(8)	10(5)	7(1)	5(2)	8(2)	4(1)	6(1)	4(2)	7(1)	11(2)	7(2)				
Y	6(10)	7(2)	14(2)	8(1)	2(1)	10(1)	11(3)	7(1)	2(1)	11(4)	8(1)	6(1)			
Z	7(1)	10(1)	5(1)	8(4)	7(1)	12(1)	9(1)	17(1)	11(1)	11(1)	7(1)	12(1)	12(2)		
Z'	6(10)	11(2)	10(1)	8(2)	4(1)	6(4)	9(1)	9(1)	9(1)	8(2)	9(2)	9(3)	3(1)	12(1)	
Z''	11(10)	19(4)	12(1)	12(1)	18(2)	2(2)	6(3)	5(2)	10(1)	4(1)	8(1)	4(1)	8(1)	15(1)	14(1)

Figures in brackets (1st batch) indicate the days after emergence the first batch is laid. Figures in brackets in other columns indicate the days between successive batches. Other figures indicate number of eggs in a batch.

Details of the oviposition of the 1st and successive batches till 15th batch, are recorded in table 2 From this table it is clear that in some batches less than twelve eggs are laid, hence it is presumed that Egg_1 of all the ovarioles do not mature simultaneously Therefore, the oviposition may be regarded as partial. Still in other cases more than twelve eggs in a batch are laid, which shows that not only one but two or more eggs are ovulated from a single ovariole. From this it may be concluded that the rate of development of the ovarioles differs from one another It is, however difficult to observe a continuous fall in number of eggs in successive batches and in no case an ovariole is observed ovulating a single egg, not even at the close of the reproductive cycle.

In table 3 a record of the interval between successive batches is shown. It varies from 1 0 to 2 0 days.

After ovulation a colourless corpus luteum is formed at the base of each ovariole.

Anatomy of the Corpus Luteum

After each ovulation which generally occurs sometimes before the oviposition the follicle shrinks and contracts quite rapidly forming a corpusluteum, which is devoid of any pigmentation From the oviposition and also from the histological picture described later it is evident that the corpus luteum at the base of each ovariole is not formed by the collapse of a single follicle but two or more follicles (Singh, 1954) This gradually contracts from 0 24 mm to 0 15 mm and eventually it is reduced to an insignificant mass. When the second batch of eggs is laid the corresponding corpus luteum is formed over the remains of the first.

Histology of the Corpus Luteum

In a fully developed egg the mature egg follicle shows the breaking down of the nuclear material into small basophilic grannules while the nuclear membrane remains distinct. Tunica propria measures 0 002 mm. The basal plug which has become very weak by this time and whose cells assume stretched and compressed form, breaks up allowing the eggs to pass down into the pedicel. After the discharge of a batch of eggs the proximal end of the follicle of the undischarged egg becomes the basal plug

As two or more eggs are ovulated almost simultaneously a very characteristic compound corpora lutea of several collapsed follicles is formed (Singh, 1954) When the eggs are discharged the follicle cells lose their cellular nature and disintegrate into deeply basophilic nuclei and cytoplasm. Within twenty four hours after ovulation the compound corpora lutea of three egg follicles is a tubular body measuring 0 24 mm, showing a narrow compressed lumen (Fig 2) The tunica propria becomes thickened (0 0032 mm) and at the basal end it telescopes and gets folded. One day after oviposition the corpora lutea contract to 0 18 mm and the lumen becomes indistinct except traces of the

passage of the discharged eggs. Whole structure appears as a mass of cytoplasm having few scattered weakly basophilic disintegrated nuclei. The tunica propria gets more folded on either sides and thickened to 0 006 mm (Fig 3) Two days after oviposition most of the cytoplasm is absorbed and only a few scattered weakly basophilic granules are seen It now measures 0 15 mm and the tunica propria further thickens to 0 0068 mm (Fig 4)

On the third day second batch of eggs is laid which passes through the remains of the 1st, very much reduced corpora lutea and a second corpora lutea of several follicles is again formed in a similar manner as described earlier in the first (Fig 5)

TABLE 3

Showing the average interval between successive batches

Between batches	1–2	2–3	3–4	4–5	5–6	6–7	7–8
Days (average)	2 1	1 3	1 5	1 3	1 6	1 8	1 6

Between batches	8 9	9–10	10–11	11–12	12–13	13–14	14–15
Days (average)	1 1	1 8	1 6	1 5	1 3	1 5	1 0

DISCUSSION

Bonhag and Wick, 1953 in *Oncopeltus fasciatus* Singh, 1954 in *Dysdercus fasciatus* have reported acrotrophic ovarioles and ovulation and oviposition both in batches. In *Bagrada cruciferarum* also, the authors have noted acrotrophic ovarioles and ovulation and oviposition in groups. Singh & Nayar 1961 have studied *Coccinella septempunctata* and observed acrotrophic ovarioles in it but the eggs were ovulated singly whereas the oviposition was in batches. A simple corpus luteum was formed at each ovulation. In the present investigation a compound corpora lutea is formed at the end of each ovulation as observed by Bonhag and Wick 1953 and Singh 1954 From these studies it is clear that eggs as they mature within the follicles of the ovarioles are ovulated into their respective pedicels and stored fo some time (Singh 1954) It is, therefore, presumed that compound corpora lutea are formed only when more than one egg is ovulated from each ovariole irrespective of the type of ovarioles.

SUMMARY

1 There are two ovaries each with six acrotrophic ovarioles.

2. The oviposition period of femal bugs is between 7 18 days and the number of eggs laid vary in successive ovipositions.

3 The interval between successive ovipositions is on an average one to two days.

4 After each ovulation a characteristic corpus luteum of several follicles is formed at the base of each ovariole.

5 Corpus luteum is colourless throughout.

6 Anatomy and histology of corpus luteum is described.

References

1 Bonhag, P F & Wick, J R. 1953. The functional anatomy of the male and female reproductive system of the Milkweed bug *Oncopeltus fasciatus* (Dallas), (Heteroptera Lygaeidae) *J Morph*, **93** 177 283.

2. Dasgupta, B & Ray H. N 1956. Observations on the 'Corpus luteum' and the 'NR bodies' in the Female gonads of bed-bugs. Abstract of the paper published in Proceedings of the 43rd Indian Science Congress (3) Sec. Zoo. and Ent. : 291

3. Singh, T 1954 Ovulation and corpus luteum formation in Insects. Thesis submitted for Ph. D in the University of London, pp. 75-93 (UNPUBLISHED).

4 Singh, T 1958. Ovulation and corpus luteum formation in *Locusta migratoria migratorioides* Reiche and Fairemaire and *Schistocerca gregaria* (Forskal) *Trans. R. ent. Soc. Lond* **110** (I) 1 20

5 Singh, T & Nayar J L. 1961 Some observations on the ovulation and corpus luteum formation in *Coccinella septempunctata* Linn. (Coleoptera Coccinellidae). *Agra Univ J Res.* (Sci.) **10** (I) 99-108.

6. Wigglesworth, V B. 1936. The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (Hemiptera) *Quart. J micr Sci.*, **79** : 91 121

Explanation of Figures

Fig 1 A mature acrotrophic ovariole.

BP—Basal plug
E_1—First egg
E_2—Second egg
E_3—Third egg
F_1—First egg follicle.
F_2—Second egg follicle.
GER—Germarium.
NS—Nutritive strand.
PC—Pedicel.
TF—Terminal filament.

Fig. 2. Base of ovariole within twenty four hours of ovulation

BP—Basal plug
F_2—Egg follicle of the undischarged egg
L —Lumen of the corpus luteum.
PC—Pedicel.
PG—Passage of the egg
PK—Pyknotic cell.
TP—Tunica propria.

Fig. 3. Base of the ovariole one day after ovulation.

BP—Basal plug

F_2—Egg follicle of the undischarged egg.

PC—Pedicel.

PG—Passage of the egg.

TP—Tunica propria.

Fig. 4. Base of the ovariole two days after ovulation.

BP—Basal plug.

F_2 Egg follicle of the undischarged egg

PC—Pedicel.

TP—Tunica propria.

Fig. 5. Base of the ovariole showing second compound corpus luteum over the remains of the 1st.

BP—Basal plug

F_2—Egg follicle of the undischarged egg

L —Lumen of the second corpora lutea.

PG—Passage of the egg

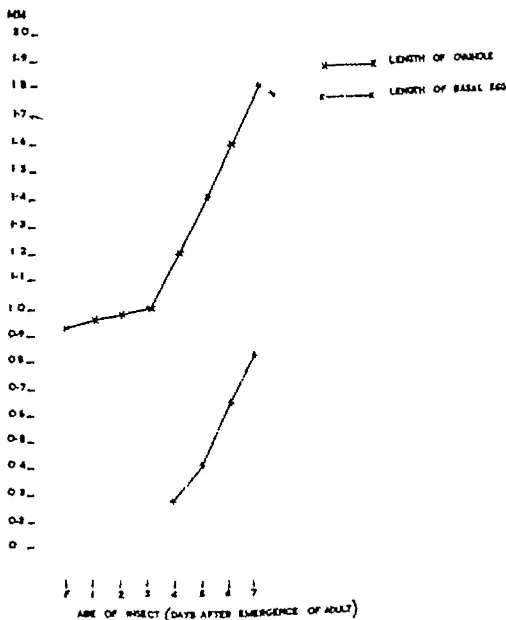
PK—Pyknotic cell.

TP—Tunica propria

TP_1 Tunica propria of 1st corpora lutea.

GRAPH 1

AVERAGE OF 5 INSECTS (SIXTY OVARIOLES)



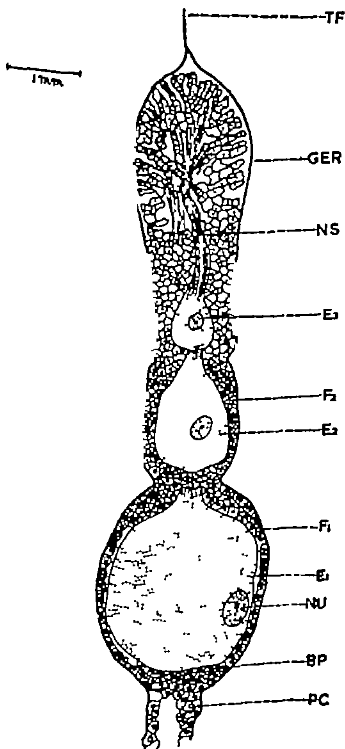


FIG. 1

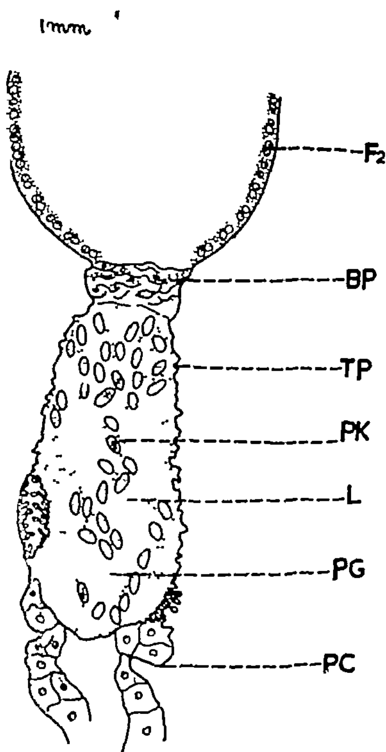


FIG 2

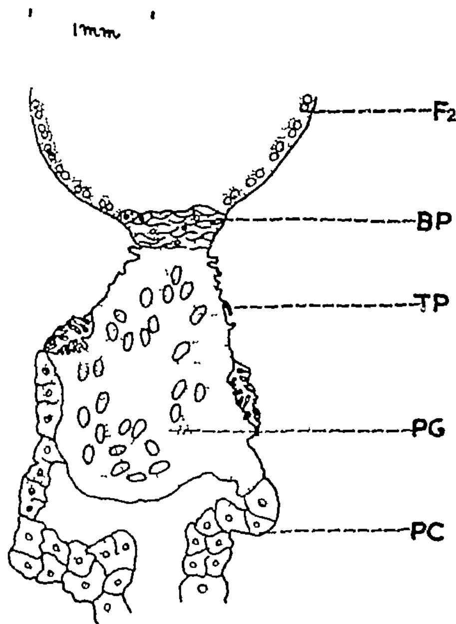


FIG 3

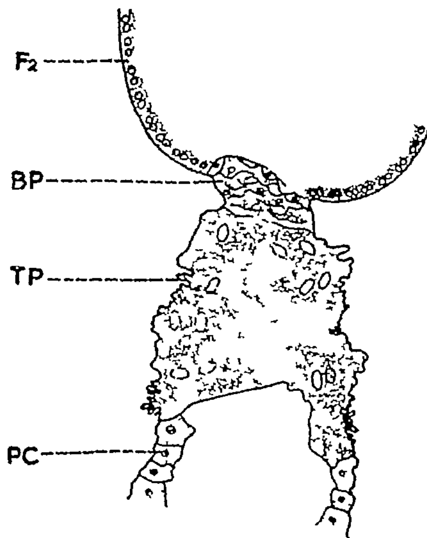


FIG 4

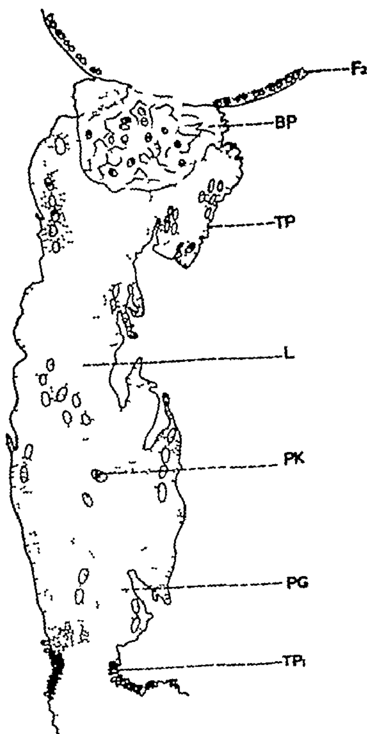


FIG 5

THE EFFECT OF AMMONIUM CHLORIDE ON THE REACTION BETWEEN CALCIUM CHLORIDE & AMMONIUM CARBONATE

SURESH SINGH
Chemical Laboratories St. John's College Agra.

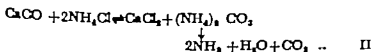
The reaction between ammonium carbonate and soluble salts of alkaline earths in the presence of ammonium chloride and ammonium hydroxide is used in analytical chemistry for the separation of these earths as insoluble carbonates from other radicals[1] and is represented by the following chemical equation —



$$CaCl_2 + (NH_4)_2CO_3 \rightleftharpoons 2\,NH_4Cl + CaCO_3 \qquad \text{I}$$

As against this practice Vogel[2] found that freshly precipitated calcium carbonate dissolves readily in cold concentrated solution of ammonium chloride and with difficulty if the precipitate has stood for 24 hours. Similar differences have been found by Warynski and Kouropatwinska[3] between aragonite and calcite in their dissolution in solutions of ammonium chloride of several concentrations. Guy Emschwiller[4] determined the solubility and solubility product of calcium carbonate in solutions of ammonium chloride and found that they are even greater than those in solutions of hydrochloric acid they assigned this behaviour to the H+ ions formed by the hydrolysis of ammonium chloride.

Cantoni and Goguelia[5] established that actually a chemical reaction takes place between calcium carbonate and ammonium chloride in solution according to the following equation —



$$CaCO_3 + 2NH_4Cl \rightleftharpoons CaCl_2 + (NH_4)_2CO_3$$
$$\downarrow$$
$$2NH_3 + H_2O + CO_2 \;.. \qquad \text{II}$$

and it goes to completion at the boiling temperature of water Barium and magnesium carbonates were found by them to react with ammonium chloride in the same manner This consideration is supported by the experiments of Shelud'KO and Cherinkov[6] who found that the decomposition of ammonium chloride goes to completion within two hours when a mixture containing solids of ammonium chlorid and calcium carbonate is heated to 300°C at 350°C the reaction gets completed in 30 minutes.

Two important points arise from th above account of the work done on this subject —

1 The precipitation of calci m carbonat on mixing solutions of calcium chloride and ammonium carbonate will be suppressed by the presence of ammonium chloride.

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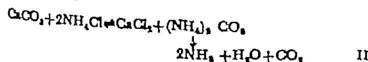
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1 The precipitation of calcium carbonate on mixing solutions of calcium chloride and ammonium carbonate will be suppressed by the presence of ammonium chloride.

amount expressed in milligrams, of Ca^{++} ions found in the solution of each reaction mixture was calculated and plotted against the amount of NH_4Cl in the reaction mixture. Some of the graphs obtained are shown in Fig 1

All graphs show that the amount of Ca^{++} ions in solution increases as the amount of NH_4Cl in the reaction mixture is increased. This shows that increased amounts of NH_4Cl bring about greater suppression of the precipitation of $CaCO_3$ in a given mixture. The graphs also show that the suppression takes place in two stages. In the first stage the amount of Ca^{++} ions in solution increases almost linearly as the amount of NH_4Cl is increased upto 3.0 3.5 grams. The curves then flatten out appreciably and once again rise in the second stage fairly rapidly at first and then slowly as the amount of NH_4Cl is further increased.

These graphs also show that the amount of $(NH_4)_2CO$ in the reaction mixture influences the precipitation of $CaCO_3$. Half an hour after mixing the constituents of the reaction the amount of Ca^{++} ions in solution in reaction mixtures containing 3.0 ml. of $(NH_4)_2CO$ are less than those in which 2.5 ml. of $(NH_4)_2CO_3$ are used, although the amount of NH_4Cl is the same in both mixtures. This shows that in the presence of a given quantity of NH_4Cl the suppression of the precipitation of $CaCO$ is decreased as the amount of $(NH_4)_2CO_3$ in the reaction mixture is increased

The aforesaid observations can be quantitatively explained on the application of the law of mass action to the reaction I which is evidently a reversible one. The equilibrium constant K of this reaction is given by

$$K = \frac{[NH_4Cl]^2}{[CaCl_2]\,[(NH_4)_2CO_3]}$$

where the terms in brackets represent the concentrations of the reactants and the products. This relation brings out that

(1) for constant value of $[(NH\ )_2CO_3]$ the value of $[CaCl_2]$ and hence of Ca^{++} ions would increase as $[NH_4Cl]$ is increased and

(2) for constant value of $[NH\ Cl]$ the value of $[CaCl_2]$ and hence of Ca^{++} ions would decrease as $[(NH_4)\ CO_3]$ increases.

Further since the amount of Ca^{++} ions in solution [illegible] amount of $(NH_4)_2\ CO$ in the reaction mixture is decreased [illegible] to dissolve by reacting chemically with NH Cl it can [illegible] that complete precipitation of $CaCO_3$ [illegible] ill not take [illegible] chloride and ammonium ca bonate are mixed in stoich[illegible]

The graphs in Fig 1 also show that increase [illegible] increase in the mount of Ca^{++} ion in a given [illegible] decreases the extent of suppression of precipitation [illegible] favours the back eaction

The influence of the total volume of the reaction mixture on the reaction was also studied. 5·0 ml. of $CaCl_2$ solution and 3 0 ml. of $(NH_4)_2CO_3$ solution of the same concentrations as used previously were mixed in a number of clean dry conical pyrex glass flasks and 3·424 g. of NH_4Cl and different volumes of redistilled water were added to the reaction mixture. The amount of reaction after 30 minutes at 85°C was determined by the same technique as followed in previous experiments. The results obtained are given in Table 1 in which 'X' represents the amount of Ca^{++} ions in mg formed in the solution and 'V' the total volume of the reaction mixture in ml.

TABLE 1

Total volume in ml. of reaction mixture (V)	Ca^{++} ions in mg produced in the whole soultion (X)	$\frac{X}{V}=K$
100	9 89	0 09890
125	11 01	0 08808
150	14 77	0 09845
175	16 93	0 09677
200	21 28	0 10640
		Mean=0 09772

It will be seen from the above Table that the amount of Ca^{++} ions in solution, that is, the extent of the reaction between $CaCl_2$ and $(NH_4)_2CO_3$ also depends upon the total volume of the reaction mixture. The amounts of Ca^{++} ions in solution increase with the increase in the volume of the reaction mixture and as shown in the last column of the Table, there is a direct proportionality between the two The proportionality constant which is nearly equal to 0·1 indicates that the amount of Ca^{++} ions in all reaction mixtures used in this Table is 1/10th of the volume of the reaction mixture

Further work on the subject is in progress. The work done by the author on the kinetics of the reaction between ammonium chloride and calcium barium strontium and magnesium carbonates will form the subject matter of other papers.

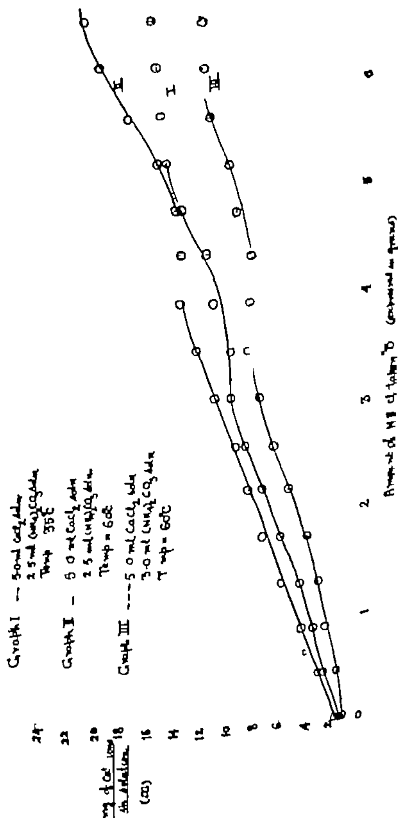
ACKNOWLEDGMENTS

The author desires to express his thanks to Dr Mata Prasad, D Sc., F R I C. F N I., for suggesting the problem and guidance throughout the investigation The author also feels grateful to Dr P I Ittyerah

Ph. D (Agra) Ph. D (Cantab) for providing the necessary facilities at the Chemical Laboratories, St. John's College Agra.

References

1. R. M. Caven, Systematic qualitative analysis, 3rd Edition 1942 pp. 136, Blackie & Sons Ltd.
2. Vogel, *Journ. Prakt Chem.* 7 455 1836.
3. T Warynski & S. Kouropatwinska *J Chim. Phys.*, 14, 328-335 1916.
4. Guy Emschwiller & Gaston Charlot, *Compt. Rend.*, 206 115-117 1938.
5. Cantoni & Goguelia *Bull. Soc. Chem.* 3 31 282, 1904; 33, 13, 1905
6. M. K. Shchul'KO & A. I Cherinkov *J Applied Chem U S. S. R.* 29 769-773, 1956


Graph I — 5·0 ml CaCl2 soln
2 5 ml (NH4)2CO3 soln
Temp 35°C
Graph II — 5 0 ml CaCl2 soln
2 5 ml (NH4)2CO3 soln
Temp = 60°C
Graph III ---5 0 ml CaCl2 soln
3·0 ml (NH4)2CO3 soln
Temp = 60°C
I
II
III
0
1
2
3
4
5
6
2
4
6
8
10
12
14
16
18
20
22
24

A CLINICO PATHOLOGICAL STUDY OF THE CASES OF INTESTINAL STENOSIS

R. S. KANCHAN
Medical College, Nagpur

Intestinal Stenosis is quite a common clinical entity Though the problem has been probed extensively yet there are many questions which remain unanswered and are more troublesome in general and clinical practice than in operation theatres. One of the main question is how to distinguish the various stenotic conditions like tuberculosis from other ulcertative granulomatous lesions of the bowel

In this study an attempt has been made to encompass the clinical features of various conditions causing intestinal stenosis and an endeavour has also been made to reconcile the etiology and pathogenesis of these conditions.

Narrowing of the lumen of the bowel can be due to two perfectly distinct causes. The stenosis is either due to changes in the bowel wall itself or the changes are outside and beyond the actual intestinal wall.

On the above basis it can be classified as follows —

I *Strictures of the bowel wall*

(a) Congenital

(I) Atresia and Stenosis

(iI) Imperforate Anus

(b) Acquired

(i) Traumatic Blunt injury abdomen and bad Surgical technique.

(ii) Inflamatory

This group includes the largest number of conditions, which are Tuberculosis, Dysentery Typhoid, Syphilis, Amyloid disease Endometriosis, Actinomycosis and Lymphogranuloma venereum of known and specific etiology and regional ileitis, jejuno ileitis, ulcerative colitis, Regional and hyperplastic colitis, Boeck Sarcoidosis, Talk granuloma and Diverticulitis of debatable etiology

(iii) Vascular

Mesenteric thrombosis Strictures after deep X-ray and reduction of Strangulated hernia.

(iv) Neoplastic.

This is an bstract of M. S. (Surgery) Thesis of the Agra University

2 *Obturator*

Gall stones, Foreign body enteroliths, Intestinal Parasites and Meconium Ileus.

3 *Compression from without*

Especially pelvic and retroperitoneal growths. The last two types are not the real stenotic lesions of the bowel.

The present study comprising of 45 cases of intestinal stenosis includes 30 cases (66 6 %) of tuberculosis, 9 cases (20%) of regional ileitis and 6 cases of non specific granuloma. Hoon *et al* (1950) in their study of benign granulomas of the ileocaecal region found 63 6% cases to be suffering from tuberculosis, 18.1% from regional enterocolitis and a similar percentage from non tuberculated enterocolitis.

Harris (1952) observed that the rarity of recorded cases of tuberculous strictures is rather surprising as the annular nature of ulceration should make stenosis a theoritically common occurrence and all modern text books of pathology stress this possibility

Intestinal stenosis is more common in women in 2nd and 3rd decade of life. Most of the cases of tuberculous and regional Ileitis are of the same sex and belong to same age group In the present study the sex ratio between males and females was 1.3.5 35 of all cases studied forming 77 7% were females.

It is a chronic disease of poor class amongst whom under-nutrition and insanitary living conditions prevail The influence of the type of diet on the incidence of intestinal stenosis is not clearly understood. 91 1 / cases of the present series were vegetarians, may be that type of vegetarian diet which an average Indian consumes, does not fulfil the basic minimum requirements of vitamins, proteins and other essentials of diet, leading to a chronic progressive type undernourishment.

It runs a chronic course and in quite a few cases the duration of symptoms may be more than three years. 68.8 / cases of the present series saught medical advice within 3 years of the onset of symptoms.

Loss of weight was reported by 44 4% cases of this study It is assumed that the loss of weight may be due to undernourishment, fear for taking food due to abdominal pain and the chronic obstructive enterogenous toxemia. Evening rise of temperature was reported by 16.6% of the tuberculous cases and 97.5 / cases were of the weak general constitution

Chronic abdominal pain diarrhoea or constipation and borborygmi are the chief complaints, and tenderness, rigidity abdominal mass and visible peristalsis are the important physical findings. Pain might be the only complaint in an otherwise healthy individual It was usually intermittent colicky in nature and mostly situated in the right iliac fossa or round about the umblicus. The pain is probably due either to hypermotility and spasm of intestinal muscles arising from local irritation of nerve endings of Meissner s plexus or to stenosis or to localised peritonitis.

Bowel disturbances in the form of diarrhoea, constipation or alternating constipation and diarrhoea were present in 57.7% cases of this series. 35.5% cases suffered from nausea and/or vomiting. Flatulence, distension and abdominal mass was also present in some cases.

Combination of anaemia and raised E. S. R. is the usual hematological finding in these cases. 80% of the total cases had anaemia and in 17.7% cases eosinophil were found to be increased. E. S. R. was found to be raised in all the cases of tuberculosis and in 4 out of the remaining 15 cases. In 88.8% cases stool and urine was found to be negative and in no case was the sputum positive for acid fast bacilli. Examination of sputum for tubercle bacilli is especially significant in view of the etiologic relationship of swallowed positive sputum to intestinal tuberculosis.

The value of radiological examination in differentiating various granulomatous conditions has not been observed. In 8 cases radiological examination did not reveal any abnormality. Out of the 77.7% cases in which the chest X-ray or Screening was done only 3 cases had positive evidence of either active or healed pulmonary tuberculosis. The absence of lesion in the lungs on X-ray examination does not rule out intestinal tuberculosis.

The commonest site of stenosis is the ileocaecal region. In the present series 62.2% cases had a lesion in the lower ileum and 35.5% in the caecum. 3.3% of the total tuberculous cases had the envolvement of pylorus, which is reportedly rare. Another uncommon site for tubercular strictures is the duodeno jejunal junction and in the present series two such cases have been observed. Hoon stated that duodenojejunal junction like pylorus is another place of natural stasis, which is a factor in localization of enteric tuberculosis. One case with a granulomatous mass and stricture in the middle of the transverse colon has also been observed.

The importance of previous use of antitubercular drugs in changing the histological picture has been stressed by Anand (1956) and Wig *et al* and in the present series too it has been established that the use of such drugs may change the histological picture in tuberculosis to that of intense fibrosis and absence of caseation but they can not introduce the typical giant cells of Crohn's disease. 50% cases of present series with history of use of antitubercular drugs grouped as non specific granuloma has some significance as Menon (1950) during his observation on animal experimentation felt that the healing tuberculosis in its terminal stages, may present a picture of non specific ulceration. Caseation, if present first, disappears after the treatment. Acid fast staining, if positive first, becomes negative afterwards.

Cases of tuberculosis had the typical microscopic appearance with the characteristic follicles consisting of caseation necrosis giant cells, epitheloid cells and lymphocytes. Although majority of the cases had this picture but in some there was evidence of commencing fibrosis with gradual disappearance of the typical characters, even than in such cases there was some recognizable

evidence to suggest tuberculosis. The picture in cases of regional ileitis was not so orderly the giant cells had different characters being irregular vacuolated and scalloped and necrosis, if present, was fibrinoid in character No caseous necrosis was observed. In all these cases there was evidence of lymphatic block resulting in reticulo endothelial hyperplasia. Cases which were labelled as non specific granuloma had a varigated picture and presented a varied appearance of either a chronic granuloma, chronic inflamation or non specific ulceration In none of these cases there was evidence to suggest either tuberculosis or regional ileitis. The granulomas usually had lymphocytic and plasma cell infiltration and were not regularly arranged

Clinically and pathologically the two conditions—hyperplastic ileocaecal tuberculosis and regional ileitis have many things in common and they mimic each other very closely and their differentiation is indeed very difficult. Microscopically the presence of giant cells in granulomatous lesions provide the main source of difficulty in distinguishing the two diseases, tuberculosis and regional ileitis. The granulomatous lesion in Crohn's disease have a disordered appearance with indistinct cell boundries, the giant cells are scalloped and irregular in size and shape and the endothelial cells are mixed with lymphocytes.

Taylor observed that in a proportion of cases of hypertrophic ileocaecal tuberculosis the lesion may show no evidence of a tuberculous etiology and a chronic tuberculous lymphangitis may be the basis of many cases of regional ileitis. He also reported that the finding of tubercle bacilli in tuberculous lesions is not always easy and a negative result by no means exclude the diagnosis of tuberculous pathology Animal inoculation too may be negative because of either an attenuated tubercle bacilli or the increased tissue resistance.

In the present study culture for acid fast bacilli was not positive in any case and only 5 of the total inoculated guinea pigs died of tuberculosis. The diagnosis in all the cases in the present series have been confirmed by histological examination of either the mesenteric lymph nodes or the piece of intestine or both The superiority of guinea pig inoculation over the culture examination has also been stressed.

The commonest complication in cases of intestinal stenosis is chronic type of intestinal obstruction, being present in 62.2% cases of the present series. Acute obstruction was present in only 15.5 / of the reported cases.

Perforation was observed in one case each of tuberculosis and non specific granuloma The reported incidence of perforation in tuberculous cases is very low being 3 7% in series of Brown and Sampson

Intussusception and Fistula formation was also observed in two cases each. Though fistula formation is a common occurrence in cases of regional ileitis but in the present series only one case had such complication

Surgical treatment is required in almost all the cases. Although excision of the affected segment of bowel is the treatment of choice but it may not

be possible to carry out this procedure in all the cases. In such cases short circuiting operations play an important role in the amelioration of the symptoms and improvement of the general condition. In view of the improvement rendered by the Surgical interference it will be advisable to open the abdomen in every suspected case than to linger on with the medical treatment.

References

1. Anand, S. S. 1956 : *Ann. Roy Coll. of Surg* 19 : 205-222
2. Brown & Sampson, *Am. Rev Tub* 1919-20 III 690 723.
3. Hoon, J R., Dockerty M. B. & Pemberton, J D 1950 : *Surg Gyn Obst*, 91 (5) : 417-440
4. Harris, W C. 1952 : Tubercle Lond. 32 (2) : 50-52.
5. Taylor A. W 1943 : *Br J Surg* 33 178-181
6. Wig, K. L., Gupta, S. P Chitkara, N L. and Manchanda R.L. 1956 : *Ind. J Tub* 3:105-110.

mum moisture (20% to 25%) to prevent any possibility of wilting due to dryness of the soil.

All the experiments were conducted in a glass house to avoid any contamination whatsoever

Observations

Field Observations The disease starts appearing in the last week of December when the plants are only a few inches above the ground and if attacked in that stage they die and completely dry out soon Those which are not affected in the young stage remain apparently healthy throughout January but in February the disease spreads The following different stages of infection have been observed —

(i) Entire plants wilting in early stages.

(ii) Entire plants wilting after flowering

(iii) Half the plant wilting in early stages as in (i) and the rest remaining healthy and producing pods and seeds

(iv) Half the plant keeps healthy throughout and the remaining part wilts after the flowering stage without producing pods or sometimes producing pods but not the seeds.

The first visual symptom of the disease is a stage of sagging of shoots followed by drooping of leaves Mostly the drooping starts from the base and finally the whole plant wilts. The yellowing of the leaves is the next apparent symptom the leaves turning brown from the margin inwards and the tip downwards. Vein clearing was observed before the plant actually wilted although some of the plants actually wilted without showing any clear sign of this symptom When the disease is in the acute form the plants bear little leaflets very closely resembling symptoms in some virus infected plants.

Symptoms in Roots The roots of infected plants are usually dark in colour and more or less devoid of laterals. Dark streaks or patches may be found below hypocotyl The diseased plants can be easily pulled out of the soil. Infection of the host occurs through roots, the fungus penetrating fairly deep to the vascular tissues the extent of infection depending on the organisation of the mycelial unit Thereafter further mycelial growth is restricted to the conducting elements of xylem (Fig 1) These findings are in accord with that of Virgin and Walker (1940) Mycelium spreads in the roots and upto stem and in many cases it eventually reaches the petioles. In still more advanced cases all the xylem elements and some of the cortical parenchyma are found to be invaded by the hyphae.

Progress of Symptoms in Shoots The obvious disease symptoms in the shoot appear two to four weeks after the plants are inoculated In laboratory conditions the symptoms were almost similar as in the fields. In the glass house infected plants showed sagging followed by drooping The phenomenon of vein clearing was also observed and even more distinctly than in field one

tions. Afterwards the wilting becomes evident and the shoots become brown and finally dry up. The oldest leaves wilt first and successively the younger ones become symptomatic in an orderly manner (Plate 1). The vascular bundles become discoloured and mats of hyphae are seen there.

The progress of vein clearing followed by yellowing of leaves in a gram plant, fifteen days old, and infected with *Fusarium orthoceras* var. *ciceri* was studied. A diagrammatic representation of the same is given in (Fig. 2) in which each circle indicates an aggregate of 10 $_{/0}$ leaves. The entire plant is involved in about four weeks' time.

Number of Chloroplasts in Healthy and Diseased Leaves. As stated above chloroplasts are adversely affected in the infected plants and this effect can be noticed much before the actual wilting. For some time plants growing in the infested and noninfested soil show little difference in their intensity of green colour but as the host plant comes under the effect of the wilt the leaves show yellowing.

The number of green chloroplasts in the healthy as well as affected leaves was counted in the region of mesophyll in fresh hand cut sections of leaves collected at about 2 p.m. after a good part of days exposure to the sun. Leaves samples were collected at random from the infested and noninfested pots ten days after the growth of the seedlings. Chloroplast counts were made in four leaflets and twenty five readings were taken in each thus making 100 observations.

TABLE 1

Average Number of Chloroplasts in one Mesophyll Cell

(Mean of 100 observations)

Day of observation after the inoculation date.	Number of Chloroplasts in healthy leaf cell.	Number of Chloroplasts in diseased leaf cell.
10	17 8	17 9
11	17 0	17 2
12	17 9	17 0
13	18 2	17 0
14	17 4	16 3
15	18 0	15 4
16	17 9	15 2
17	17 8	14 7
18	17 6	13 5
19	18 4	12 0
20	18 2	10 0
21	18 0	6 0

From Table 1 it is apparent that the loss of chlorophyll is distinct in the plants of the infested pots

Starch Test Starch tests have been used in the case of virus diseases to trace the path of systemic infection (Holmes 1932 and Smith 1934) Kalyansundaram (1954) has employed this starch test in the case of *Fusarium* wilt of cotton. In similar tests performed here the leaves were plucked from healthy and infected plants late in the evening and tested for starch. The leaves from a healthy plant gave a characteristic dark blue colour while those from the affected plant gave a weak positive test The test was also extended to the leaves showing the phenomenon of 'vein clearing' The region of vein clearing' gave a negative test for starch.

Symptom Production with reference to the age of the Host Plant For this experiment the plants were infected at three different periods of their growth.

(i) Seeds germinated in infested soil.
(ii) Plants inoculated when 10 days old.
(iii) Plants inoculated when 20 days old.

The plants of different age groups were inoculated by transplanting the healthy plants, which were previously sown in sterilized garden soil to the infested soil The results of symptoms production are recorded below

TABLE 2

Showing Symptom Production with reference to age of the Host Plant

No of days after the inoculation	Seeds germinated in infested soil	10 day old plants transplanted to infested soil	20 days old plants transplanted to infested soil
10	No symptoms	No symptoms	No symptoms
15	Early vein clearing	No symptoms	No symptoms
21	Vein clearing	Early vein clearing	No symptoms
27	Yellowing followed by browning	Vein clearing	Early vein clearing
35	Complete wilting	Yellowing followed by browning	Vein clearing
45	——	Complete wilting	Yellowing followed by browning
59	——	——	Complete wilting

After complete wilting the plants begin to dry up. It is seen in table 2 that with advance in age of the host plant at the time of infection the length of time taken for the appearance of the symptoms and complete death increased. The rate of progress of the disease is inversely proportional to the age of the host plant at the time of infection.

Discussion

'Vein clearing' noticed in the leaves of gram infected with *Fusarium orthoceras* var. *ciceri* was the earliest symptom of wilting as seen by unaided eye. Similar observations were made by Foster (1941) and Kalyanasundaram (1954) in tomato and cotton wilt respectively. The progression of 'vein clearing' in host plant (Text Fig. 2) seems to indicate the probable path of toxin movement inside the vascular strands. The symptoms were seen to develop in the leaves in acropetal succession supporting the findings of Braun *et al* (1951) and Kalyanasundaram (1954). Some of these symptoms also resemble those of deficiency diseases as pointed out by Lockhart (1939). The number of chloroplasts decreases in the mesophyll cells of the leaves in diseased plants much before the leaves show yellowing. Foster (1946) and Kalyanasundaram (1952, 1954) have also indicated reduction in chlorophyll and also the number of chloroplasts as a symptom of *Fusarium* wilt. Yellowing, drooping and curling of the leaves begin from the lower leaves and extend upward (Text Plate I). The crown then droops and complete wilting occurs followed by death of plants. Kalyanasundaram (1954) states that there is inhibition of starch synthesis along the path of toxin and in the advance stages of the disease when the stem and petiole get necrosed the normal photosynthetic activity of the leaves ceases.

The results of experiments dealing with the effect of the age of host indicate that younger plants are more readily susceptible than the older ones, the latter taking a longer period to wilt. In older plants the fresh weight increases and also the absolute synthesizing capacity which are factors that mainly affect the reaction of the toxin (Gaumann 1951).

In field condition it has been noticed that although host plants are susceptible at all stages of maturity but the symptoms production is related to their age, and also to the intensity of the disease. Further the phenomenon of partial wilting a characteristic of vascular wilts (Butler 1918) has also been observed in this case. On the basis of these symptoms the author has developed a technique to appraise the disease (1960).

Summary

Investigations on wilt of gram (*Cicer arietinum* L.) caused by *Fusarium orthoceras* App. & W. var. *ciceri* Padwick in relation to its symptomatology were made. The fungus attacks the host plants at different age levels. Partial wilting, a characteristic phenomenon of vascular wilts was also observed. Presence of hyphae and spores was detected in xylem vessels of the affected plants. 'Vein clearing' was noticed as the first visible symptom of the disease. This symptom develops in acropetal succession. It was observed that chloroplasts

are adversely affected in the infected plants and this fact can be noticed much before the actual wilting Starch formation is also similarly affected. Leaves from healthy plants gave a characteristic dark blue colour in Sach s iodine test while those from affected plants gave a weak positive test. The extent of wilting appears to be influenced by the age of the plants at the time of infection.

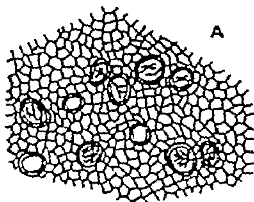
ACKNOWLEDGEMENTS

The author is very much grateful to Professor S Sinha, Head of the Department of Botany Agra College, Agra for his guidance and keen interest in this work throughout the course of investigations. He is also thankful to the authorities of B R. College Bichpuri Farm, Agra for their kind facilities.

REFERENCES

1 Bawden, F C. 1943 : Plant viruses and virus diseases. Chronica Botanica, Waltham Mass.
2 Brian, P W *et al* 1951 Uptake of antibiotic metabolites of soil micro-organisms by plants. *Nature* 167 : 347 349
3 Butler, E. J 1918: Fungi and diseases in plants. Thacker Spink & Co. Calcutta.
4 Chauhan S K. 1960 : A technique for disease appraisal and crop loss relationship in *Fusarium* wilt of gram (*Cicer arietinum* L.) *Agra University Jour Res.* (*Science*) 9 (2) 253-260
5 Foster, R.E. 1946 : The first symptom of tomato wilt : Clearing of the ultimate veinlet in the leaf. *Phytopath.* 36 697-698
6. Gaumann E. 1951 Some problem of pathological wilting in plants. *Advanc Enzymol.* 11:491
7 Holmes, F O 1932 : Movement of mosaic virus from primary lesions in *Nicotiana tabacum*. *Cont. Boyce Thompson, Inst.* 4 297 322.
8. Kalyanasundaram, R. 1952 : Ascorbic acid and *Fusarium* wilted plants. *Proc Indian Acad Sci. Sect. B* 36 (3) : 102 104
9 Kalyanasundaram R. 1953 Soil conditions and wilt diseases in plants. Fungal wilt and changes in host metabolism. *Ph. D Thesis Madras Uni.* (*Seen J I B. S.*)
10. Kalyanasundaram, R 1954 Soil condition and root diseases. XIII Symptomatology of *Fusarium* wilt. *Jour Indian Bot. Soc* 33 (4) : 329-337
11 Linford, N B. 1931 Transpirational history as key to the nature of flowing in *Fusarium* wilt of pea. *Phytopath.* 21 (8) : 791 796.
12 Lockhart C. I 1959 : Symptoms of mineral deficiency in the Lowbush Blueberry Plant Disease Reporter 43 (1) January 15, 1959.
13 Padwick, G W 1940 The genus *Fusarium* III A critical study of the fungus causing wilt of gram (*Cicer arietinum* L.) and the related species in the subsection *orthocera* with special relation to variability of key characteristics. *Indian Jour Agri. Sci.* 10 (3) 241 284
14 Pierson, C. F *et. al.* 1955 Histological studies on the role of pectic enzymes in the development of *Fusarium* wilt symptoms in tomato. *Phytopathology* 45 (10) 524-527
15. Sattanarayan G & Kalyanasundaram R. 1952 : Soil condition and root diseases. V Symptomatology of wilted cotton and red gram. *Proc Indian Acad Sci. Sect. B* 36 5-8.
16. Smith, K. M. 1934 Some aspects of plant virus problem. *Agri. Progress* 11 85-92.
17 Vaughn, W J & Walker, J C. 1940 : Relation to the near wilt fungus to the pea plant. *Jour Agri. Res* 60 241 248.
18 Wellman, F L. 1941 Epinasty of tomato, one of the earliest symptoms of *Fusarium* wilt. *Phytopathology* 31 : 281 283.

Root Xylem



L Power



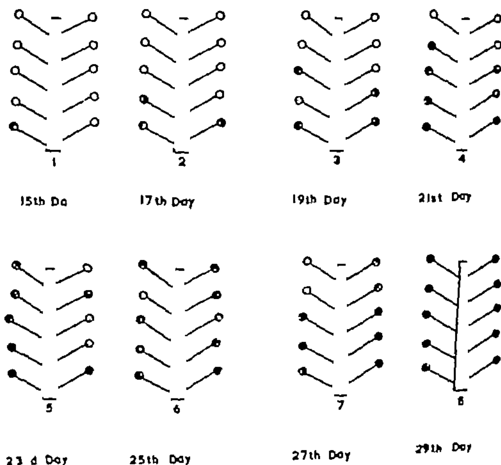
H Power

Showing Fungus hyphae in vessels

FIGURE 1

FIGURE 2

REPRESENTS PERCENTAGE OF LEAVES SHOWING THE DIFFERENT CATEGORIES OF INFECTION



O Healthy

⊕ Early Stage Of Vein Clearing

O Clear Symptoms Of The Same

● Advance Stage Of Vein Clearing Followed By Yellowing

PLATE I

EFFECT OF SOIL MOISTURE ON ROOT ROT OF GUAR (*CYAMOPSIS PSORALIOIDES* DC.) AND WILT OF GRAM (*CICER ARIETINUM* L.) CAUSED BY *SCLEROTIUM ROLFSII* SACC.*

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INTRODUCTION

Garrett (1944) has given a consolidated list of diseases favoured by low or high moisture content of the soil Most wilt diseases caused by species of *Fusarium* are favoured by high soil moisture (Goss 1921 and 1923, Clayton 1923, Tharp and Young 1939 Strong 1946, Subramanian 1950 and Chauhan 1950) Among the diseases favoured by low soil moistures are seedling blight of wheat and corn (Dickson *et al* 1923) potato scab (Sanford 1923) tomato wilt (Foster and Walker 1947) and *Fusarium* disease of broad beans (Yu and Fang 1948)

In this paper results are presented on the effect of soil moisture on root-rot of guar (*Cyamopsis psoralioides* DC.) and wilt of gram (*Cicer arietinum* L.) both caused by *Sclerotium rolfsii* Sacc.

METHOD AND MATERIAL

The experiments were conducted in pot cultures Four moisture levels viz., 10, 15 20 and 25% were maintained on oven dry basis of soil and ranged well within the water holding capacity of the soil, which was determined following Keen-Raczkowski's method as described by Piper (1944)

Six pounds of garden soil was filled in pre weighed glazed metal pots (8½ × 7") The inoculum of the pathogen was raised on autoclaved corn-meal-sand medium and equal quantities of the same were mixed thoroughly with the soil in experimental pots seven days before sowing Five replications (5 pots) were maintained for the infested series and only one pot for the control in each treatment. Six seeds were sown per pot after seven days of infestation The experiments were performed in a glass house. Th moisture levels were kept constant by weighing each pot every day and dding water to maintain the original weight Observ tions on percentage of seedling emergence and of post-emergenc seedli g mortality were ecorded 4 days fter sowing for 44 days at regular intervals of 4 days Th data obtai ed were analysed statistically following nalysis of variance method The pathogen was reisolated from the affected colla region of the plants to make sure the cause of their death due to the parasit Th seedlings in the control pots remained healthy throughout the period of investigation i each treatment

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* A part of Thesis approved for the Ph. D. degree of the Agra University Agra.

Results

Root-rot of Guar

Observations recorded on percentage germination post-emergence seedling mortality and total percentage mortality (pre and post-emergence together) in root rot of guar are presented below and results discussed.

Table 1

Percentage Emergence of Seedlings and Percentage Pre-Emergence Mortality in Guar

Moisture levels	Emergence of Seedlings: Control series	Emergence of Seedlings: Infested series	Pre-emergence mortality
10%	100 00	80 01	19 99
15%	100 00	63.34	26 66
20%	100 00	93.34	6.66
25%	100 00	96 67	3.33

The germination of guar was 100% in the control series in all the four moisture levels but was reduced in the infested series more so at low soil moisture levels. This is indicative from the percentage emergence of seedlings at various levels (table 1) Thus pre-emergence loss of the host was maximum at 15 % followed by at 10% moisture level the mortality figures being 26.66 and 19 99% respectively At higher levels the mortality is considerably reduced Heavy mycelial infection over the surface of ungerminated seeds was noticed under low soil moisture levels.

Table 2

Progressive Increase in Percentage Post Emergence Seedling Mortality in Guar

Days after sowing	Soil Moisture Levels: 10 %	15%	20%	25%
4	0 00	0 00	0 00	0.00
8	0 00	0 00	0 00	3.33
12	13 34	3.33	6.67	6.67
16	13.34	16 67	13.34	6.67

(Continued on page 297)

TABLE 2—(*Contd.*)

Progressive Increase in Percentage Post Emergence Seedling Mortality in Guar

Days after sowing	Soil Moisture Level 10%	15%	20%	25
20	26 67	26.67	20 00	16.67
24	26 67	26.67	23.33	23.33
28	43 34	36.67	30 00	26.67
32	43.34	46 67	30 00	26.67
36	46 67	50 00	30 00	26.67
40	46.67	50 00	30 00	26.67
44	46 67	50,00	30 00	26.67

Total post-emergence seedling mortality was the highest (50.0%) in 15% moisture level, and it is less both in lower as well as in higher moisture values. Considerable reduction in seedling mortality occurs at 20 and 25% soil moisture, the mortality figures being 30 00 and 26.67% respectively. The rate of mortality is likewise affected by moisture being more rapid in lower percentages (10 20%) than in 25% moisture. The observations indicate an intimate relationship of mortality with percentage moisture level and age of the host plant. The mortality increased progressively for 28-32 days after sowing, but afterwards no mortality occurs except in 15% moisture in which it continues upto 36th day. Thus the plants more than 28-32 days old are not susceptible but favourable moisture conditions may prolong the susceptibility for another 4 days.

TABLE 3

Percentage Mortality (Pre & Post Emergence) in Guar (Mean of 5 pots, each with 6 seeds)

Moisture Levels 10%	15%	20%	25%	C. D. at 5% level
60 67	76 67	36 67	29 33	15.62

The total percentage mortality is 66.67 at 10% level and it reaches the maximum value of 76 67 at 15% level although there is an evident increase in mortality but the increase is not statistically significant (difference being less than C. D) Thus the two moisture levels (10 and 15%) can be grouped together for their effectiveness in causing mortality In higher soil moistures the total mortality decreases significantly but there is no significant difference between 20 and 25% moisture levels. Hence the four soil moistures can be arranged in the sequence of reducing total mortality and simultaneously grouped in the following way —

Moisture level	Moisture level	Moisture level	Moisture level
15%	10%	20%	25%
76 67	66 67	36.67	29.99

C D is 15 62

WILT OF GRAM

The influence of different soil moistures on pre and post-emergence mortality and also total percentage mortality in gram is discussed below

TABLE 4

Percentage Emergence of Seedlings and Percentage Pre Emergence Mortality in Gram

Moisture levels	Emergence of Seedlings		Pre-emergence mortality
	Control series	Infested series	
10 /	100 00	66.67	33.33
15	100 00	83.33	16.67
20%	100 00	96 67	3.33
25%	100 00	93 36	6.67

Table 4 shows a reduction in the emergence of seedlings in the infested series both in lower as well as higher soil moisture levels. At low soil moisture levels the infected seeds were found to be profusely enveloped in a fungal mat of the parasite while in high soil moisture there is a rapid rotting of the diseased seeds and unemerged seedlings. Thus pre-emergence mortality of the host is highest (33.33%) at 10° moisture and it decreases subsequently with rise in moisture content of the soil

TABLE 5

Progressive Increase in Percentage Post Emergence Seedling Mortality in Gram

Days after sowing	Soil Moisture 10%	15%	20%	25 %
4	0.00	0.00	0 00	0.00
8	0 00	0.00	3.33	0 00
12	3 33	16 67	3.33	0 00
16	20.00	23 33	10.66	3 33
20	46.66	33.33	16.66	6.67
24	56.66	36.67	20 00	6 67
28	60 00	40 00	20 00	13.33
32	60 00	53 33	33 33	20.00
36	60 00	56.67	40 00	20.00
40	60.00	56.67	40 00	20 00
44	60 00	56.67	40.00	20.00

In gram post-emergence seedling mortality was most favoured by low moisture, being maximum (60%) at the end of 44 days in 10% moisture level. At 15% soil moisture it is only slightly less but in 20 and 25% soil moisture the mortality figure is considerably reduced as was observed for guar The mortality rate is evidently more rap d in 10% moisture and decreases as the moisture level is increased.

Unlike guar gram plants under less favourable moisture conditions (15-20%) remain susceptible for a longer period (36 days) although th total mortality reached is less This probably brings in another unknown factor into play

TABLE 6

Total Percentage Mortality (Pr & Post-Emergence) in Gram
(Mean of 5 pots each with 6 seeds)

Soil Moisture 10%	15%	20%	25 %	C. D at 5% level
93.33	73.33	43.33	26.66	17.29

The above table clearly shows that the maximum total percentage mortality (pre and post-emergence) occurs at 10% soil moisture and it decreases progressively in high soil moisture levels. This shows that by keeping the soil moisture high there is a significant reduction in total percentage mortality The order of reduction in percentage mortality being 10% (93 33) < 15% (73.33) < 20 % (43 33) < 25% (26 66)

Further there is significant difference in the reduction of total, mortality at 10% and 15% soil moisture levels C. D being 17 29 and mortality figures were 93.33 and 73.33% respectively But there is no significant difference between total mortality at 20 % and 25% soil moistures, the respective mortality values being 43.33 and 26.66% Thus the four soil moisture levels under observation can be placed under three groups.

Moisture level	Moisture level	Moisture level	Moisture level
10%	15%	20%	25%
93 33	73.33	43 33	26 66

C. D is 17.29

DISCUSSION

These two diseases like a number of other soil-borne diseases seem to be favoured by low moisture content of the soil Dickson *et al* (1923) noted greater development of seedling blight of wheat and corn due to *Gibberella saubinetii* in drier soils and attributed it to retarded host resistance. Sanford (1923) obtained badly scabed potato tubers in dry soils. Similarly Foster and Walker (1947) observed that tomato (variety Bonny Best and Marglobe) wilted in dry soil and very wet soil decreased their potential susceptibility Yu and Fang (1948) found that dry and medium moist soils were better suited to *Fusarium* disease of broad bean than wet and saturated soils.

St eets (1948) gave a passing remark that *Sclerotium rolfsii* was injurious to guar in wet soils but furnished no details. While Luttrell (1951) describing the diseases of guar in Georgia, stated that the same fungus (*S rolfsii*) caused exceptionally high mortality of the host plants in dry season Epps, Patterson Freeman (1951) while studying the physiology and parasitism of *Sclerotium rolfsii* found that the parasite was highly virulent and attacked seeds of sugar beet, garden pea rice and soyabeans in soils having a moisture content much lower than that required for their normal germination.

Guar attacked by *Rhizoctonia solani* and *Fusarium caeruleum* resulting in root-rot and wilt respectively is reported to show greater disease incidence in low soil moi ture than in higher moisture level (Singh and Singh ——— The

host reacted in a similar way with *Sclerotium rolfsii* at different soil moisture levels employed in the present investigation, the maximum mortality of 70.67% occurring at 15% soil moisture and it decreased considerably in high soil moisture levels.

Similarly wilt of gram caused by *Sclerotium rolfsii* is also favoured by low soil moisture The maximum mortality (93.33%) occurring in 10% moisture level while in 15-25% moisture the mortality decreases from 73.33 to 26.66 It is interesting to note that wilt of gram caused by another parasite i.e. *Fusarium orthoceras* var. *ciceri* is favoured by high soil moisture (Chauhan 1959)

The rate of seedling mortality is similarly affected in both the diseases under study lower moisture levels accelerate the mortality while in higher levels the rate of mortality is slow A perusal of tables 2 and 5 indicates that the mortality is also related to the age of host plant greater percentage mortality occurring in seedling stage and ceases after a certain period depending upon the percentage moisture of the soil

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REFERENCES

1 Chauhan, S. K. 1959 Studies on wilt of gram and crop loss estimates. *Ph. D Thesis, Agra University, Agra (India)*

2. Clayton, E. E. 1923 : The relation of soil moisture to the *Fusarium* wilt of tomato. *Amer J Bot* 10, (3) 133-146.

3 Dickson J G., Eckerson, S. H. & Link, K. P 1923 The nature of resistance to seedling blight of cereals. *Proc. Nat. Acad Sci. Washington* 9 434-439

4 Epps, W M. Patterson, J C & Freeman I. E. 1951 Physiology of parasitism of *Sclerotium rolfsii Phytopath.* 41 (3) 245-256.

5. Foster R. E. & Walker J C. 1947 Predisposition of Tomato *Fusarium* wilt. *J Agric. Res.* 5-6 165-185

6. Garrett, S. D 1944 Root Disease Fungi. Waltham Mass. U S. A. Chronica Botanica Publication.

7 Goss R. W 1921 Temperature and humidity studies of some *Fusaria* rots of Irish potato. *J Agric. Res.*, 22 (2) 63-85.

8. Goss, R W 1923 Relation of environment and other factors to potato wilt caused by *Fusarium oxysporum*. *Agric. Exp. Sta. Nebrask Res. Bull.* 23 84.

9 Luttrell, E. S. 1951 Diseases of Guar in Georgia. *Plant Dis Reptr* 35, (3) 166.

10. Piper C S 1944 Soil Plant Analysis Inter Science Publishers, Inc New York 63

11 Sanford G. B. 1923 The relation of soil moisture to the development of common scab of potato. *Phytopath.* 13 231-236.

12. Singh, Babu & Singh, R. S 1956 Temperature and moisture relations of the fungi causing seedling-rot, root-rot and wilt of *Cyamopsis psoraloides* DC. II Effect of soil moisture on mortality under controlled conditions. *Agr Uni. Jour Res (Sci.)* 5 (1) 139-141

13 Streets, R. B. 1948 : Disease of Guar (*Cyamopsis psoralioides* DC.) *Abstr. Phytopath.* 38 (11) : 918.

14 Strong, M. C. 1946 : The effect of soil moisture and temperature on *Fusarium* wilt of tomato. *Phytopath.* 36 (3) : 218-225.

15 Subramanian, C. V. 1950 : Soil conditions and the wilt diseases in plants with special reference to *Fusarium vasinfectum* on cotton. *Proc. Ind. Aca. Sci.* 31 : 67-102.

16. Tharp, W. H. & Young, V. H. 1939 : Relation of soil moisture to the *Fusarium* wilt of cotton. *J. Agric. Res.* 58 : 47-61.

17 Yu, T. E. & Fang, C. T. 1948 : *Fusarium* Disease of Broad Bean. II. Further studies on Broad Bean wilt caused by *Fusarium avenaceum* var. *fabae*. *Phytopath.* 38, (5) : 331-342.

PHYSICO-CHEMICAL STUDIES ON α-NITROSO β-NAPHTHOL AND ITS CHELATE COMPOUNDS*

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The principal object of the present work was to investigate in detail the physico-chemical characteristics of 1-nitroso-2-naphthol which is used as an analytical reagent in qualitative and quantitative estimation of number of metallic ions. This utility of the substance appeared primarily due to the existence of the nitroso and hydroxyl groups in 1 2 position in the naphthalene ring, leading to the formation of stable chelate complexes with metallic ions. No quantitative data are available in the literature on the dissociation constant of OH group or on the stability constant of the complexes, which are of marked importance in understanding the various processes occurring in and development of, analytical procedures.

The Thesis consists of two parts containing in all eight chapters.

In part I of the thesis are reported detailed investigations on spectrophotometric determination of the dissociation constant of OH group in 1-nitroso-2 naphthol. The low solubility of the compound in aqueous medium set a limitation to the use of potentiometric studies on the determination of dissociation constant of 1-nitroso-2 naphthol. The use of recent developments of spectrophotometric methods enabled the author for the first time in the field of research to determine accurately the dissociation constant (k) of 1-nitroso-2-naphthol in aqueous solutions (Chapter 2). Detailed investigations showed that the data were not amenable for analysis by conventional methods of spectrophotometric determination of k. Equations for possible existence of dimerization and absorption by both anion and the undissociated molecule of 1-nitroso-2-naphthol were deduced. These showed the absence of dimerization due to intermolecular complex formation in 1 nitroso-2-naphthol. The extinction coefficients of dissociated and undissociated molecules of 1-nitroso-2-naphthol were determined. These data enabled the author to compute the dissociation constant k which corresponded to 1 288 × 10^{-} at 30°C. The thermodynamic dissociation constant K was evaluated by employing the modified Debye and Huckel formula. K was found to be markedly dependent on temperature (T) of the system (Chapter 3). It obeyed the following relationship

$$-\ln k = (-1561/T) + (33\ 22) + (-3.005\times10^{-[illegible]})T$$

from this, the various thermodynamic functions associated with the dissociation of 1 nitroso 2-naphthol were evaluated for the first time at different temperatures. At 30° C these were as follows :

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*This is an abstract of the thesis submitted and approved for the Degree of Doctor of Philosophy by the Agra University, Agra in ... 1961

$$\Delta F = 46470 \text{ I Joules/mole}$$
$$\Delta S = 9879 \text{ I Joules/mole}$$
$$\Delta H = -123.7 \text{ I Joules/deg/mole}$$
$$\Delta C_p = 153.3 \text{ I Joules/deg/mole}$$

The dependence of K on the dielectric constant of the medium was investigated over a wide range of conditions (Chapter 4) using different solvents such as C_2H_5OH CH_3OH and CH_3COCH_3 etc. The following equation due to Wynne Jones was found to be applicable

$$\Delta \ln k = -\frac{e^2}{2kT}\left(\frac{1}{\gamma_{H^+}}-\frac{1}{\gamma_{A^-}}\right)\Delta\left(\frac{1}{D}\right)$$

In accord with this equation pK varied linearly with $\frac{1}{D}$ the slope of these plots gave a value of 0 7398 Å 0 7398 Å and 0 7396 Å as the radius of anion of I nitroso-2 naphthol in ethyl alcohol, methyl alcohol and acetone respectively Infra red spectrophotometric analysis of 1-nitroso-2 naphthol (Chapter 5) showed that in accord with the data reported in chapter 2 of this thesis, I nitroso-2-naphthol did not exist in dimer form. Conclusive evidence was, however obtained for its existence as internally chelated substance due to the formation of intra molecular hydrogen bonding The mechanism of the dissociation of I nitroso-2-naphthol was discussed in detail.

In part II of the thesis are presented data on determination of the stability constants of metal chelate complexes of 1 nitroso-2-naphthol using the pH metric method due to Bjerrum. The inapplicability of other methods was discussed. Bjerrum's method envisaged the use of dissociation constant K of I nitroso-2-naphthol which was determined and reported in part I The solubility of these complexes necessitated the use of 50 per cent alcoholic solutions for the above method the value of dissociation constant under these conditions are reported in chapter 4 The stability constants K of different metal ions such as Co^{2+} Pb^{2+} Ni^{2+} Cu^{2+} Ca^{2+} Mg^{2+} etc. were determined The bond energies of these complexes were evaluated Copper and lead chelates appeared to be more stable than those of other metals. The values of K were correlated with the position of the metals in the periodic table and the charge and radius of the metal ions. These considerations showed that orbital electron of the metal ion was involved in the chelate formation.

At the end of the thesis was given an Appendix which reported for the first time detailed investigations on polarographic reduction of 1-nitroso-2 naphthol at the dropping mercury electrode. The polarographic characteristics such as $E_{\frac{1}{2}}$ and diffusion current constants $\left(\frac{id}{C} m^{\frac{2}{3}} t^{\frac{1}{6}}\right)$ were computed. The variation of $E_{\frac{1}{2}}$ with pH was linear in accord with the data obtained by other workers with other organic substances; these results showed that hydrogen ion was involved in the reduction process. Det[illegible] [illegible]tions on the determination of diffusion coefficient under polaro[illegible]

using McBain and Dawson cell showed that the number of lectrons involved in the reduction of 1-nitroso-2 naphthol at the dropping mercury electrode was one, contrary to the stoichiom tric requirement of two hydrogen atoms for the reduction of 1 nitroso-2 naphthol to hydroxylamine der v tive of 2-naphthol. A mechanism applicabl for polarographic conditions was suggested.

PATHOLOGICAL ANATOMY OF THE FLORAL PARTS AND FRUITS OF CORIANDER AFFECTED WITH *PROTOMYCES MACROSPORUS* UNG

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The tumour disease of coriander caused by *Protomyces macrosporus* Ung is widely prevalent in the eastern districts and Bundelkhand region of Uttar Pradesh and also in the northern districts of Madhya Pradesh. The disease causes a distinct loss in yield mainly by producing hypertrophied fruits which may not be set at all when the parasite infects at the flowering stage. Hypertrophied fruits in addition to losing their condiment value, do not germinate. The author (1954) estimated the mean loss per plant to be about 15% with a mean total disease intensity of 23%. The present piece of investigation was undertaken to trace the changes brought about in the affected floral parts and fruits by the disease.

Method and Material

The material for the present study was collected from infected as well as from healthy plants of coriander from fields. Buds and fruits of different ages were fixed in F. A. A. and dehydrated in grades of alcohol and embedded in paraffin. Sections were cut at 8-20 μ and stained with Heidenhain's iron-alum haematoxylin or safranin and fast green. Both the stains gave satisfactory results. Fruits were also macerated in 5% KOH for six hours at 40°C. After several washings with tap water they were transferred to lactic acid for 2 hours and then stored in 70% alcohol for dissection. Germination tests were made in sand to see the viability of the seeds.

Observations

The inflorescence of *Coriandrum sativum* L. is a compound umbel, made up of 5-8 umbellets. In the same umbellet all buds or fruits may be infected or some may remain healthy (Figs 1 & 2). The changes brought about by the parasite largely depend upon the developmental stage at which infection reaches.

Effect upon flowers and buds When infection reaches very young buds, they become blighted and later fall off from the plants within a week or so. Teased preparations of buds reveal that the entire tissue is filled up with chlamydospores.

The relatively older buds, when severely infected show the presence of chlamydospores of the fungus in all the floral parts (Figs 3, 4, 5 & 7). The petals shrivel up accompanied with compression of locules and suppression

A part of the Ph. D. thesis approved by the Agra University Agra.

of ovules (Figs 5 3 & 4) All the five or fewer stamens may be infected. The pollen sac of the infected lobe in the affected anthers is completely compressed resulting in degeneration of the pollen grains (Figs 8 & 9) The endothecium is not well marked as is the case in healthy anther lobes (Figs 9 & 12) When part of the anther is healthy the pollen grains of the unaffected lobes look exactly like those of completely healthy stamens (Figs 6 & 7) Akai (1937) also observed compression of pollen sacs and degeneration of pollen in the affected anthers attacked by *Albugo candida* and called this phenomenon as "oppressive atrophy" caused due to the pressure rising from hypertrophy of anther cells. It appears that the same effect results in atrophy of pollen sacs in coriander infected with *Protomyces macrosporus* Ung

When the infection is mild *i.e.* it has reached only the stalk of the flower or when formation of chlamydospores is restricted only to a small part of the ovary wall the ovule developes. The embryo sac, endosperm and embryo are all formed (Figs. 13 14 15 & 16)

Effect on fruits The chlamydospores of the fungus ultimately are found in all parts of the fruits viz. pericarp, stylopods, carpophore and testa (Figs. 17 & 22) In fully hypertrophied fruits variations in size of pericarp, carpophore locule testa and endosperm were observed (Table 1)

TABLE 1

Variations in size of the various parts of fruits in coriander

(Mean of 10 values)

Fruits (mm)	Pericarp B	Carpophore B	Locule L	Locule B	Testa L	Testa B	Endosperm L	Endosperm B
Healthy	0 2	0 3	4 0	1 2	3 7	1 0	3 7	1 0
Fully hypertrophied	1 1–1 3	1 3	7 1	1 3	3 1	0 7	—	—

L Length B Breadth

The pericarp and the carpophore increase in size, and the locule increases almost to double in hypertrophied fruits the testa on the other hand shrinks along the breadth while the endosperm is altogether missing

Pericarp In fully hypertrophied fruits the pericarp loses the distinction of ridges and grooves (Figs 23 & 24) The various layers of the pericarp also lose their identity and all its cells look alike (Figs 25 & 26) The epidermal cells elongate periclinally (Fig 26) In transverse section they elongate in tangential direction and the radial dimensions of the cells are shorter than those

of the normal ones (Figs. 28 & 29) Akai (1937) also made similar observations in epidermal cells of stem of rape invaded by oospore stage of *Albugo candida* The angular sclerenchyma so prominent in healthy mature fruits cannot be recognized in the diseased ones (Figs 25 & 26) The parenchymatous cells enlarge and take an irregular shape The innermost layer of the pericarp made up of narrow radial elongated thick walled cells, is hardly recognizable The xylem elements are not so compact as in the healthy fruits and probably the elements appear stretched in width. On the whole the total number of cells and the size of the cells of the pericarp increase accompanied with enlargement of intercellular spaces.

The oil vittae lying close to the outer layers in the pericarp in healthy fruits appear to lie in deeper tissues presumably due to increase in outer cell layers. The boundary of the cavity of vittae is well marked in healthy fruits but is not at all distinct in hypertrophied fruits. In partly infected fruits (Figs 19 & 20) changes in the tissue of pericarp appear only in the vicinity of infection.

Carpophore and stylopods In the region of carpophore also there is a general increase in size of cells. The oil ducts localised in this zone are completely absent (Figs. 23 & 24) The longitudinal splitting of the carpophore characteristic of a healthy ripe fruit does not take place in hypertrophied fruits as a result of which there is no separation into two mericarps. A similar type of increase in cell layers and cell size is observed in stylopods and the stalk of diseased fruits.

Testa In partly infected fruits the testa appears almost normal (Fig 27) but in fully hypertrophied fruits it gradually shrivells and is finally reduced to a small crumpled-up envelope lying towards the micropylar end of the locule (Figs 22 & 36)

Endosperm In fully hypertrophied fruits the endosperm is lacking (Fig 24) This fact is confirmed by microscopic examination of serial sections of the fruits as well as their dissections. But in partly diseased fruits (Fig 19) where infection has reached very late probably after endosperm and embryo formation, the cells of endosperm show increase in size (Figs 32, 33 & 34) and also formation of some kind of granular matter In the fruits where infection is heavy and chlamydospores are formed in the entire fruits (Fig 21) the endosperm appears to lose its form. The cytoplasm of the cells form a peripheral scanty layer with less prominent nuclei. The granular matter which appears in partly infected fruits is completely lacking (Fig 35) Presence of chlamydospores in the endosperm is not seen either in partly or fully infected fruits.

Embryo In a fully hypertrophied fruit, as revealed by microscopic examination and dissection the embryo is completely lacking (Fig 22) Dissection of mature but a relatively less hypertrophied fruits show the presence of poorly differentiated embryos (Figs 37 39) The size of embryos in such fruits, as

compared to healthy embryos is very small. In partly infected fruits where chlamydospores are confined to the pericarp and infection has reached very late, a normal embryo is present. Germination tests show that fully hypertrophied fruits or relatively less hypertrophied ones do not germinate. But partly infected fruits, in which normal embryo is seen germinate like the healthy ones.

From these studies it appears that the increase in size of the affected fruits and buds is largely due to the enlargement of cells of the affected tissues (hyper trophy) increase in size of the intercellular spaces and also increase in number of cell layers (hyperplasy) It is difficult to locate a general division of cells in the affected tissues although some cell divisions could be seen in the pericarp and the stalk region of diseased fruits (Figs 30 & 31) The walls of the affected cells appear to be thin and delicate probably because the cells keep pace with enlargement of the protoplast by stretching, but no addition of new wall substances take place by intussusception or apposition as suggested by Akai (1951) The cytoplasm gets restricted only to the peripheral zone of the cells and the nuclei less prominent.

Kuster (1910 1911 and 1925) classified plant galls into two large groups, organoide (abnormal formation of organs) and histioide (abnormal tissue formation) The latter was further distinguished by him into kataplasmatic (differentiation of plant tissue of low degree) and prosoplasmatic (complicated tissues differing entirely from those in normal condition) Following his concept of classification the galls formed by *Protomyces macrosporus* on fruits of *Coriandrum sativum* can easily be placed under the prosoplasmatic type of histoide galls The observations are in line with the study of galls on *Crepis japonica* and *Lactuca debilis* infected by *Protomyces Inouyei* and *Protomyces Lactucae debilis* (Akai 1939) respectively

Summary and Conclusions

The changes in buds, flowers and fruits depend chiefly upon the stage at which the infection takes place. Young buds, when severely infected, become blighted and fall off In relatively older buds the petals shrivel up accompanied with compression of locules and suppression of ovules Affected anthers show compression of pollen sacs and degeneration of pollens. When infection is mild and the formation of chlamydospores is restricted to a small portion of the ovary wall, the normal development of the ovule continues.

In fully hypertrophied fruits the distinction into various layers of pericarp is lost The epidermis elongates periclinally and the sclerenchymatous layer lacks altogether The dorsal ... lie deeper and lose their distinct shape A great increase in size of the carpophore is seen and the oil ducts are absent.

The testa shrivels up while the endosperm and the embryo are lacking in hypertrophied fruit In partly infected fruits when the infection reaches late the endosperm is formed but the size of its cells increases and granular matter

in the cell appears Less hypertrophied fruits show presence of shrivelled endosperm with poorly differentated embryos. The fully or relatively less hypertrophied mature seeds do not germinate but partly infected seeds are viable.

The chlamydospores are found in the sepals, petals, anthers, and ovary wall of buds and flowers also in the pericarp carpophore, stylopods and testa of fruits. The increase in size of affected fruits is mainly due to the enlargement of cells of affected tissues (hypertrophy) increase in size of intercellular spaces and also increase in number of cell layers (hyperplasy)

ACKNOWLEDGMENTS

The author expresses his grateful thanks to Professor S Sinha, Head of the Botany Department, Agra College, Agra for his guidance and critical suggestions in preparation of the manuscript. His sincere thanks are also due to Dr Y D Tyagi, Lecturer Saugor University for his assistance in morphological interpretations and drawings

REFERENCES

1 Akai, S. 1937 : Ueber die pathologische Histologie des von *Albugo candida* (Pers.) O Kuntze verursachten hypertrophischen Rapsstengels. Forsch. d. Gebiet d. Pflanzenkrankh 3 71-83.

2. Akai, S 1939 : On the anatomy of *Protomyces* galls of peduncles of dandelion. *Jour Japanese Bot.* 15 84-86.

3. Akai S. 1951 Studies on the pathological anatomy of fungus galls of plants. *Mem. Coll. Agr., Kyoto Univ.* 58 1-60

4 Gupta J S. 1954 Disease appraisal of stem-gall of *Coriandrum sativum* L. *Indian Phytopath.* 7 53-60

5 Kuster E. 1910 Ueber organoide Gallen. *Biol. Zentralbl* 30 116-128.

6. Kuster E. 1911 Die Gallen der Pflanzen 84-170. Leipzig.

7 Kuster E. 1925 Pathologische Pflanzenanatomie. 184-501 Aufl. 3 Jena

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Explanation to Figures

Figs. 1-2. Fig 1 Two infected Inflorescences showing infection at the flowering stage. Fig 2, Inflorescences with healthy and diseased fruits.



Fig 1

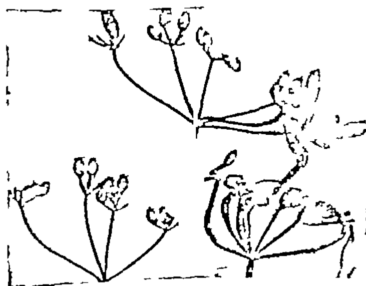


Fig. 2

EXPLANATION TO FIGURES

Figs. 1 Fig. 1 Two infected inflorescences showing infection at flowering stage. Fig. 1 florescences with healthy and diseased fruits.

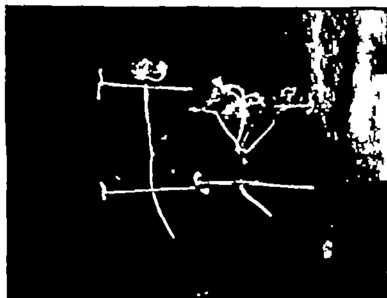
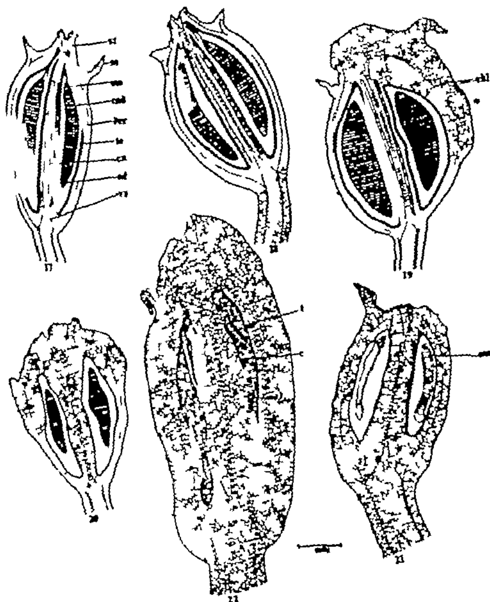


Fig 1



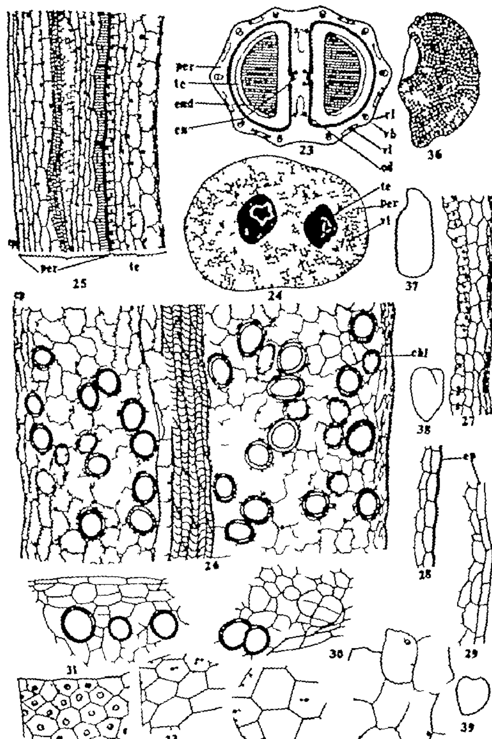
Fig 2

Figs. 17-22 : Fig 17 Healthy fruit. Fig 18, Fruit showing infection in the stalk and the base of one of the mericarps. Fig 19 Fruit showing infection in the stylopods and pericarp of one side only (partial hypertrophy) Fig. 20, Fruits showing infection in the stylopods, carpophore and the upper parts of the pericarp of both the mericarps. Fig. 21 Hypertrophied fruit with endosperm shrivelling up. Fig 22 Hypertrophied fruit showing absence of endosperm and embryo and reduced testa with chlamydospores.


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Figs. 23-39. Fig 23 Semidiagrammatic T S of a mature healthy fruit showing ridges and grooves, ittae testa, pericarp endosperm and vascular bundles (X 12) Fig 24, T.S. of a hypertrophied fruits (X 12) Fig 25, A portion of the pericarp and testa of a healthy fruit showing different cell layers (X157) Fig 26 A portion of the pericarp of a fully hypertrophied fruit. (X 157) Fig. 27 A portion of the testa of a partly infected fruit (X 157) Fig. 28 & 29 Few cells of epidermis of healthy and hypertrophied fruits respectively (X 233) Fig 30, Cells showing division in periclinal plane in the upper region of the pericarp (X 133). Fig. 31 Cells showing division in periclinal plane in the stalk of the diseased fruit (X 133). Fig. 32, cells of endosperm of a healthy fruit (X 400) Fig. 33 cells of endosperm from healthy side of a partly infected fruit (X 400) Fig 34 cells of endosperm from infected side of partly diseased fruit (X 400) Fig. 35 cells of endosperm of relatively less hypertrophied fruit (X 400). Fig 36 Entire testa dissected out from the fully hypertrophied fruits (X 16) Figs. 37-39 Poorly differentiated embryos taken out from relatively less hypertrophied fruits (X 33)

Abbreviations : an : anther chl : chlamydospores; em : embryo; end nu. : endosperm nucleus; lo locule ps : pollen-sac; se sepal zy : zygote : ca : carpophore; cav : cavity; en : endosperm; od : oil duct; per pericarp; st : stylopodia; te : testa; vs : vascular supply ep : epidermis; ri : ridge vb : vascular bundle; vi; vittae; gr : granular matter


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BACTERIOLOGICAL STUDIES OF BOVINE SEMEN AS COLLECTED FOR ARTIFICIAL INSEMINATION*

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In view of lack of published data in India, on the Microbial flora of semen as collected for Artificial Insemination, a study was initiated to isolate and identify the various organisms that might be commonly encountered in the semen of cow and buffalo bulls.

One hundred and forty-five semen samples (89 from Murrah Buffalo bulls and 56 from Hariana bulls) collected by using artificial vagina, mostly from the Animal Genetics section of Mathura Veterinary College and some from Baboogarh Farm and other places, were subjected to cultural examination and the isolated organisms typed.

The isolated organisms from semen of Hariana bulls belonged to the Genera Staphylococcus, Rhodococcus, Bacillus, Corynebacterium, Proteus, Bacterium, Pseudomonas and other untyped Gram positive and Gram negative rods. In addition to the organisms of the same genera, Micrococci were also isolated to a limited extent from Murrah bulls. In Murrah bulls the occurrence of Bacillus, Bacterium, Corynebacterium Streptococcus and Pseudomonas were found in greater percentage than in Harianas, while members of coliform group were isolated in lesser frequency The percentage of isolations of Staphylococcus was more or less the same in either class of animals.

A limited isolations attempted from swab samples made from external genitalia revealed a positive correlation indicating that sheath harboured these organisms. From the sheath of Hariana and Murrah b lls the organisms isolated were Staphylococcus, Rhodococcus, Streptococcus Diphtheroids, Bacillus, Proteus, Coliform organisms, Bacterium, Pseudomonas and other untyped Gram positi e and Gram negative rods. Thus the overall picture of organisms isolated from the semen of Murrah and Hariana bulls were nearly from the same genera as from the sheath swabs. Table I illustrate the findings.

Viabl bacterial counts were made from 15 samples, which included 5 samples from 4 Murrah bulls and 9 samples from 9 Hariana bulls, while one sample from pooled semen of 8 Murrah bulls was also taken. The minimum

* This is an abstract of the thesis submitted to the Agra University for the Degree of Master of Veterinary Science in Advanced Bacteriology

TABLE 1

Table showing the percentage of various organisms in the semen or sheath of Hariana and Murrah Bulls

Sl. No.	Organisms	Percentage of occurence of organism: Semen Hariana	Semen Murrah	Sheath Hariana	Sheath Murrah
1	STAPHYLOCOCCUS	61	50	60	25
	(i) *Staph. aureus*	9 4	11 4		—
	(ii) *Staph. albus*	32 9	34 2	6	25
	(iii) *Staph. citreus*	37 6	15 2	2	12.5
2	Micrococcus	—	3 8		—
3	STREPTOCOCCUS	28 2	38	40	39 5
	(i) Animal pyogenes		3 8		1 5
	(ii) *Str acidominimus*	9 4	15 2	20	25
	(iii) *Str viridans*			20	—
	(iv) *Str uberis*		7 6		—
	( ) Enterococci	4 7	15 2		—
4	CORYNEBACTERIUM	4 7	30 4	—	—
	(i) *G. Pyogenes*	4 7	15 2		
	(ii) Non haemolytic diphtheroids		19		—
5	BACILLUS	24 5	50	60	62.5
	(i) *B. subtilis*	18 8	26 6	—	25
	(ii) *B mycoides*	14 1	22 8	40	37.5
	(iii) *B. cereus*		15 2		—
	(iv) *B sphericus*		15 2	40	— 11 5
	( ) *B megatherium*	4 8			12.5
6	UNTYPED GRAM POSITIVE RODS	9 4	22 8	—	
7	PROTEUS	23 5	19.2	40	—
	(i) *P vulgaris*	9 4	7 6		—
	(ii) *P mirabilis*	9 4	3 8	—	—
	(iii) *P species*	14 1	11 4	40	
8	BACTERIUM	37 6	15.2	20	12 5
	(i) *B coli*	18 8	3 8	20	— 12.5
	(ii) *Aerobacter aerogenes*	18 8	11 4		12 5
9	*Bacterium alkaligenes*	18 8	41 8		25
10	*Pseudomonas aeruginosa*	9 4	26 6	—	—
11	Untyped Gram negative rods.	23 5	7 6		—

bacterial count from Hariana bulls was estimated to be 166 thousand per ml., while the maximum count was 4138 thousand per ml The arithmetic average from Hariana bulls came to 1489 thousand per ml. In murrah bulls

the minimum bacterial count was 177 thousand and the maximum was 2955 thousand per ml. The average in this case came to 1773 thousand organisms per ml. of semen.

The effect of various antibiotics was studied on diluted buffalow bull semen. The antibiotics and sulphadrugs included were Sulphatriad, Sulphapyridin and Elkosin in doses of 300 mg per ml., Streptomycin 200 micrograms per ml. Penicillin-G Sodium and Penicillin (Procaine) 100IU per ml. Streptomycin 200 micrograms per ml., Aureomycin 100 millimicrograms per ml. and Terramycin 50 micrograms per ml. The combinations of some of the drugs in the same doses were also studied in their bacteriostatic action, which included (i) Streptomycin plus Penicillin, (ii) Sulphatriad plus Penicillin and (iii) Sulphatriad Penicillin and Streptomycin. All the drugs checked bacterial growth in diluted semen when stored at 5° C. from 24 hours to ten days. The combinations of Sulphatriad plus Penicillin were found to be the best in reducing the bacterial population. Amongst the single antibiotics used, Streptomycin proved the choicest.

From what has been stated above it is apparent that the practice of cleaning the sheath of bulls before collection of semen and judicious use of Sulphadrugs and Antibiotics for checking the bacterial growth during storage of semen, is highly desirable. Also it is necessary to carry out bacteriological examination of semen before it is used for artificial insemination, since many bulls, though apparently healthy may be transmitting pathogenic microbes in their semen and may cause large scale dissemination of infection.

A COMPARATIVE STUDY OF THE CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF CERTAIN INDIAN INSECTS

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A comparative study on the cytoplasmic inclusions in the oogenesis of *Attacus ricini* Boisd. and *Bombyx mori* linn. has been made in the present work. The material for the present investigation was obtained from the Government Silk Institute, Nathnagar (Bhagalpur)

The ovary in *A ricini* and *B mori* is paired. In both the insects each ovary consists of four ovarian tubules or ovarioles. In each ovariole of both *A. ricini* and *B mori* two proper zones are recognised—the zone of multiplication i.e., the end chamber or the germarium, and the zone of growth or the vitellarium The end chamber in *A ricini* is finger-shaped while in the case of *B mori* it is rounded or club-shaped. The vitellarium part of the ovariole of both *A ricini* and *B mori* shows a beaded appearance and the beads, which represent oocytes, are arranged in a linear series Further the oocytes present a progressive increase in their dimensions from the anterior end of the vitellarium to its posterior where large sized ova are present.

Each oocyte is provided with a group of nurse-cells at one pole. The maximum number of nurse-cells associated with a well defined oocyte in both *A ricini* and *B mori* has been noticed upto six (Plate I Fig 1) In the earlier stages of the growth period of oocytes the follicular cells are loosely arranged around the oocyte and they also extend in between the nurse-cells on the one hand and the oocyte on the other (Plate I Fig 1) Gradually the follicular cells form a compact epithelium around the oocyte and the follicular cells extending in between the consecutive oocyte and group of nurse-cells also gradually disappear probably by a process of dissolution The finally formed ova ready for oviposition do not have any nurse-cells, associated with them and they are also without any follicular epithelial covering

The ovarioles have also been observed to show the presence of the terminal filament. Thus the ovarioles of both *A ricini* and *B mori* are of meroistic and poly-trophic type

A consideration of the origin and relationship of the nurse-cells and follicular cells of *A ricini* and *B mori* has been made in the present study The nurse-cells, follicular cells and oocyte in both the insects, in all probability originate from the undifferentiated cells contained in the end chamber of the egg tube (Plate I Figs. 1 2)

This is an abstract of the thesis submitted and approved for the Ph. D. degree of the Agra University in the year 1960.

The presence of the Golgi elements in the follicular cells of *A ricini* and *B mori* has been noticed but the juxta nuclear and peri-nuclear Golgi elements have not been observed in the follicular cells of the two insects. The infiltration of the Golgi elements from the follicular cells to the oocyte in both *A. ricini* and *B mori* has been observed (Plate II Fig 3 Plate X, Fig 5)

The presence of the Golgi elements in the nurse-cells of the two insects has been also observed. However the occurrence of the Golgi elements in the nurse-cells of the early oocyte of *A ricini* has not been noticed but it has been marked in the case of the nurse-cells of the early oocyte of *B mori* (Plate X, Fig 3) The infiltration of the Golgi elements from the nurse-cells to the oocyte has been recorded in both *A ricini* and *B mori* (Plate III, Fig 1 Plate X, Fig 1)

The morphology of individual Golgi elements in the early stages of oogenesis of both *A ricini* and *B mori* is not clearly revealed. However when the Golgi elements become scattered or dispersed in the ooplasm, they are perceptible in respect of their morphology In *A ricini* the Golgi elements are granular crescentic and vesicular (Plate II Fig 3) and in the case of *B mori* they are as crescents dots and granules (Plate X, Fig 5)

In both *A. ricini* and *B mori* the Golgi elements of the oocyte ultimately give rise to the fatty yolk bodies by their metamorphosis, which involves a process of swelling and coalescence of the Golgi elements

A study on the mitochondria in the oocytes of *A ricini* and *B mori* has been made during the different stages of their growth in the egg tube The mitochondrial elements in the end chamber of the egg tube of both the insects have not been found to occur in the present study In the early oocyte of *A. ricini* the mitochondrial elements of dusty or cloudy appearance have been noticed (Plate IV Figs 1,2) in a juxta-nuclear location But the juxta-nuclear mitochondrial elements have not been observed in the early oocyte of *B mori* The mitochondrial elements in peri-nuclear location in the early oocyte of both *A ricini* and *B mori* have been observed (Plate IV Fig 3 Plate V Fig 4)

A cortical location of te mitochondrial elements has been noticed in *B mori* (Plate VI Fig 1) The presence of the mitochondrial elements in the follicular cells of the early oocyte of *A ricini* and *B mori* has not been observed. In later stages of the growth of the oocytes of the two insects, the follicular cells show the presence of the mitochondrial elements in them. The infiltration of the mitochondrial elements from the follicular cells to the oocytes of both *A ricini* and *B mori* has been observed.

The nurse-cells in the early oocytes of *A ricini* and *B mori* show the presence of the mitochondrial elements in them (Plate IV Fig 2 Plate V Fig 4). The infiltration of the mitochondrial elements from the nurse cells to the oocyte has been detected in both *A ricini* and *B mori* (Plate IV Fig 4 Plate V Fig. 5)

The morphology of the mitochondrial elements in the early oocytes of both *A ricini* and *B mori* is not distinctly revealed as they are of very fin form In their dispersed phase they appear granular or dot-like in form in the oocytes of *A. ricini* (Plate IV Fig. 5) and so also they are in the oocytes of *B mori* (Plate IV Fig 4)

In the advanced oocytes of both *A. ricini* and *B mori* they form clumps and ultimately metamorphose into the proteid yolk bodies

No mitochondrial element in the oocytes of either *A ricini* or *B mori* has been observed in a process of division although two closely placed mitochondrial elements giving the picture of their being in the process of division have been observed.

The nucleolus in the early oocyte of both *A ricini* and *B mori* is basophil (Plate VII Fig 3 Plate XII Fig 2) but soon, in both the cases, it becomes oxyphil. In *A ricini* two nucleoli have been observed. This double nucleolus in the early oocyte of *A ricini* is a secondary *formation. In the oocyte of B mori* the division of a single nucleolus into two has not been observed. The nucleolus in the oocytes of both the insects becomes vacuolated and gives rise to nucleoluar particles or plasmosomal bodies. These plasmosomal bodies extrude through the nuclear membrane into the ooplasm as such In both *A. ricini* and *B mori* the extruded nucleolar particles or plasmosomal bodies are always oxyphil. In th early oocytes of the two insects just a few nucleolar particles have been observed within the nuclear membrane together with the nucleolus.

In both *A ricini* and *B mori* the extruded nucleolar particles are similar in their staining reaction to the nucleolar particles within the nuclear membrane Therefore, it has been concluded that the extruded nucleolar particles in all probability *originate from the nucleolus of the oocytes of the respective insects.*

In *A ricini* a passage like streak, due to a nucleolar particle shot out from the vacuolated nucleolus, in the ooplasm has been observed (Plate VIII Fig 2) This streak in all probability has been formed due to the coincidence of the moment at which, in this particular oocyte the nucleolar particle was sh t out with the moment of fixation of the oocyte. The phenomenon of nucleola extrusion shows a decrease in the advanced oocyte of both *A ricini* and *B mori* and extruded particles finally disintegrate and get mixed up with the ooplasm.

There is absence of a classical yolk nucleus of lamellar type in both *A ricini* and *B mori* However th juxta-nuclear patch of cytoplasm w th inclusion has been observed in both the cases and that has been considered as yolk nucleus. Further the dense cytoplasmic areas (Plate IX, Figs. 5, 2 4 Plate XII Fig 4) in both the insects *have also been noticed. The origin of such areas* resembling yolk nucleus has been discussed. In *A. ricini* a yolk nucleus o

type other than the lamellar described by several oriental as well as occidental workers has been noticed (Plate IX, Fig 3)

The discussion on the origin of dense cytoplasmic areas throws light on the ultimate origin of the so-called yolk nucleus In all probability the origin of yolk nucleus and dense cytoplasmic areas is due to the streaming movement of the cytoplasm linked with its colloidal property Besides, the diffusion currents from the heamocoelic fluid entering the oocyte, and the water currents in the ooplasm also play an important role in the localisation of the cytoplasmic inclusions in the ooplasm in such a manner as to cause the formation of structure appearing like the yolk nucleus

The study on vitellogenesis of *A ricini* and *B mori* brings out clearly that two types of yolk bodies are present in the advanced oocytes of each of the two insects. One of the two types of yolk bodies is of fatty nature and the other is of proteid nature The fatty yolk is derived from the Golgi elements (Plate II Fig 5 Plate X, Fig 5) Besides, the Golgi fatty yolk, the diffusion of free fat particles or globules from the adipose or fatty tissue into the oocyte of both *A ricini* and *B mori* has been observed (Plate V Figs. 2, 3 Plate VI Fig 3) The phenomenon of diffusion of free fat particles from the adipose tissue into the oocyte has not been previously recorded by any worker on oogenesis in the studies of cytoplasmic inclusions Therefore, it is regarded that *A. ricini* and *B mori* seem to be unique in demonstrating the phenomenon of diffusion of free fat particles from the adipose tissue, surrounding the ovarioles, into the oocyte

In case of the oocyte of *A ricini* and *B mori* the above mentioned phenomenon has been observed in Zenker Helly preparations stained in iron alum heamatoxylin (Plate V Fig 3 Plate VI Fig 3) and in case of the oocyte of *A ricini* in preparations made by F W A method and stained in iron alum heamatoxylin (Plate V Fig 2)

The origin of the proteid yolk spheres in oocytes of both *A ricini* and *B mori* has been traced to the mitochondrial elements. The mitochondrial elements swell, form clumps and thus ultimately metamorphose into the proteid yolk spheres (Plate IV Fig 5 Plate V Fig 1 Plate or VI Fig 4)

The nuclear extrusions in both *A ricini* and *B mori* have not been observed to play any direct role in the process of vitellogenesis.

The presence of two types of yolk bodies—the fatty yolk bodies and proteid yolk bodies has been further confirmed by staining the contents of oocytes of *A ricini* and *B mori* with Sudan IV Sudan III and Scharlach R. also give positive results in this respect.

It has been further observed in both *A ricini* and *B mori* that the appearance of fatty yolk precedes that of the protied yolk bodies.

The investigation in the oogenesis of *A. ricini* and *B. mori* by supravital experiments has shown results in support of the view that the Golgi elements are independent cytoplasmic inclusions. The young oocytes of *A. ricini* and *B. mori* treated with neutral red solution show the presence of vacuoles and later on when they are treated with a few drops of two per cent osmic acid the Golgi elements become blackened and thus their presence is detected side by side of the red stainable vacuoles (Plate XIV Figs. 1 2 Plate XV Figs 1 2)

Further the supravital experiments in respect of the Golgi elements also reveal the morphology of individual Golgi elements. Accordingly the Golgi elements in the oocytes of both *A. ricini* and *B. mori* are polymorphic—granular vesicular and dot-like. The vesicular Golgi elements show the presence of an osmiophilic rim or externum, and the osmiophobic core or internum (Plate XIV Fig 3 Plate XV Fig 3)

The oocytes of the living pupae of both *A. ricini* and *B. mori* were dissected out and centrifuged for three hours in normal saline in an electric centrifuge capable of producing 3 000 revolutions per minute. The oocytes were next fixed and the finally prepared sections of the centrifuged oocytes after staining, showed the displacement of the cytoplasmic inclusions in both the insects. In case of *A. ricini* the fatty yolk bodies were thrown to the peripheral region of the ooplasm while the proteid yolk bodies were stratified next to the fatty yolk spheres. The layer of proteid yolk bodies also showed a few swollen Golgi elements on its outer aspect. On the inner aspect of the layer of the proteid yolk bodies a less dense mass of cytoplasm containing the mitochondrial elements, in its peripheral part and fine mitochondrial particles in its central part, was observed. The oocyte nucleus remained undisturbed. This shows that in *A. ricini* the oocyte nucleus is heavy enough to counteract a centripetal force, generated by 3 000 revolutions per minute, of an electric centrifuge. In nurse-cells the inclusions get concentrated at one point (Plate II Fig 5)

In case of *B. mori* no doubt, the cytoplasmic inclusions were separated from one another but the stratifications of different inclusions at different points in the ooplasm were not exactly similar to that of *A. ricini*. A strip of blackened particles—the Golgi elements—was observed beneath the zone of nurse-cells Next to the strip of the blackened Golgi particles was observed an appreciably large strip of the yolk bodies of mixed type—proteid and fatty yolk bodies. Mitochondrial elements were also noticed in this region An almost clear cytoplasmic area just opposite to the pole of nurse-cells was observed which contained a few Golgi elements, mitochondrial particles as well as a few yolk spheres (Plate VI Fig 4)

Like the oocyte-nucleus of *A. ricini* the oocyte-nucleus of *B. mori* also remained undisturbed and the centripetal force generated by an electric centrifuge capable of producing 3,000 revolutions per minute used for the experiment could not remove it from its normal location. Nevertheless, the centrifuge

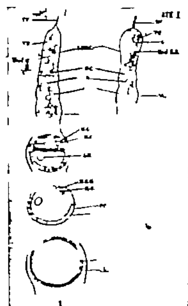
experiments in both the cases clearly prove the existence of different inclusions in respect of their gravity

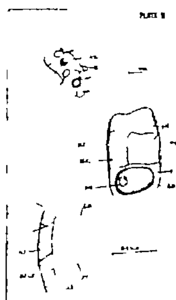
The difference in the stratification of the cytoplasmic inclusions in the centrifuged oocytes of *A. rivus* and *B. mori* may be accounted for the varied velocities gained by the cytoplasmic particles or inclusions and also due to the difference in the colloidal phases of the ooplasm at various points in the beginning of the experiment.

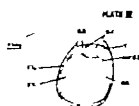
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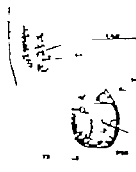
AR. A	Archoplasmic area.
C. C A	Clear cytoplasmic area.
Ch	Chorion
Conc. Inc.	Concentration of Inclusions.
D C A.	Dense cytoplasmic area.
Dl. C. N C.	Diffusion of cytoplasm from nurse cells.
Di. Nu Ex.	Disintegrating nucleolar extrusion.
F C. N	Follicular cell nucleus.
F E	Follicular epithelium.
F P	Follicular particles or globules.
F T	Fatty or adipose tissue.
F Y B	Fatty yolk bodies
F V	Fatty vacuole
G	Germarium.
G B	Golgi bodies
G C	Germ cells
INF G B	Infiltration of Golgi bodies.
INF M	Infiltration of Mitochondria
M	Mitochondria
M C.	Mitochondrial clumps.
N C	Nurse cell
N C. N	Nurse cell nucleus.
Nu.	Nucleolus.
Nu Ex.	Nucleolar extrusion
Nu P	Nucleolar particles or plasmosomal bodies.
O N	Oocyte nucleus.
O Nu	Oocyte nucleolus
P B	Proteid yolk bodies.

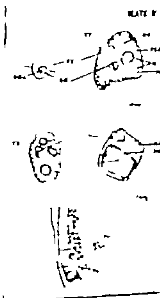
S F E C.	Sheath of flat epithelial cells.
Stp. G B.	Strip of Golgi bodies.
Stp Y B	Strip of yolk bodies.
T F	Terminal filament
T P	Tunica propria.
Und. G C.	Undifferentiated germ cells.
V M	Vitelline membrane.
V N	Vacuolated nucleus.
Vl.	Vitellarium.
Y B	Yolk bodies.
Y N	Yolk nucleus.



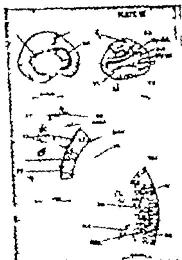


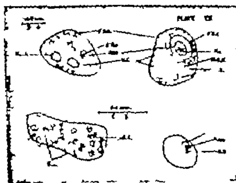


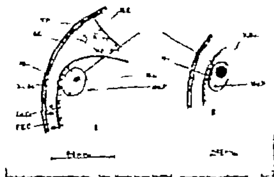


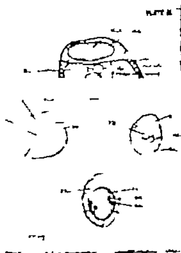












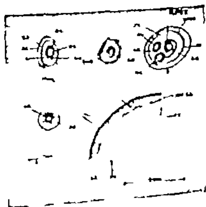


PLATE XI

PLATE IX